

The University of Chicago

A STUDY OF CERTAIN
METABOLIC EFFECTS OF TESTOSTERONE
PROPIONATE IN EUNUCHOIDS AND
IN NORMAL MEN AND WOMEN

DEPARTMENT OF BIOCHEMISTRY
1939

By
KATHRYN KNOWLTON

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THE EFFECT OF TESTOSTERONE PROPIONATE ON NITROGEN, ELECTROLYTE, WATER AND ENERGY METABOLISM IN EUNUCHOIDISM

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In a preceding study of the effects of long-continued treatment of eunuchoids with testosterone propionate (1), a surprising and consistent increase in body weight was observed, amounting from 1.5 to 2.0 kg. in 10 days and to 4.5 to 8.5 kg. in 28 to 64 days. This increase in weight was, in all 4 patients, accompanied by fulness of the face, in 1 by pitting edema of the ankles, and in 1 by puffing of the hands, indicating a gain in body water. In 2, however, an unmistakable increase in food intake was reported by the patients, and although, in 3, reduction of the dose of testosterone propionate or its discontinuance led to a rapid loss of at least part of the weight gained, in 1 only (H. K.), an insignificant loss appeared, as if tissue had been materially augmented by the course of treatment. Studies in 1 patient (N. T.), through such a period of weight gain showed a slight rise in the B.M.R. indicating that depression of the resting oxygen consumption was not of importance. Moricard and Bize (2) in boys, and Foss (3) in a eunuch, had also noticed this gain in weight with testosterone compounds, as had Usui and his co-workers (4) with a urinary androgen in 3 eunuchoids. Korenchevsky, Dennison and Brovsin (5) report somewhat lower weights for the body as a whole, liver, heart and kidneys in castrated rats than in controls and found that certain dosages of testosterone elevated these weights toward normal. They speak accordingly of an anabolic property of testosterone.

While our work was in progress, Thorn and Harrop (6) published their discovery that retention of sodium, together with associated water, may be produced in the normal dog by the administration of estrone, estradiol, progesterone, pregnanediol and testosterone, an effect like that of adrenal cortical extracts in normal dogs (7), and in normal man (8, 9). Thorn and Harrop were able to simulate the 'sodium-retaining' action of adrenal cortical extracts in a patient with Addison's disease by estrone (6), and Thorn, Nelson and Thorn (10) have given physiological significance to these important observations by the suggestion that changes in weight of women during the menstrual cycle, together with menstrual edema, are due to such action of the female sex hormones. The chemical relationships between the adrenal and sex hor-

mones that are now becoming evident (11, 12) are thus seen to have biological corollaries.

Another important metabolic influence of the androgens, that of diminishing nitrogen excretion in the castrate male dog, has been recently established through the work of Kochakian and Murlin (13, 14) and Kochakian (15). In their experience with androsterone, androstene-dione, and testosterone, this decline in urinary nitrogen was accounted for by the change in the urea fraction, and was unaccompanied by any increase in the nitrogenous constituents in the blood. The protein stored, they felt, might be due to the growth of the prostate and seminal vesicles.

With these findings in mind we have analyzed, in part, the effects of a daily

TABLE 1. EFFECT OF TESTOSTERONE PROPIONATE (25 MG. DAILY) ON CERTAIN BLOOD CONSTITUENTS OF *N. D.*

Date	Plasma proteins gm./100 cc.	N.P.N. mg./100 cc.	Urea mg./100 cc.	Urea clear.	Hb. gm./100 cc.	Rbc. m./cu. mm.	Rbc. vol. %
9/22/37		26	13.3	52			
9/24/37	7.56		15.7	40			
9/29/37	6.38	34					
10/ 1/37			13.8	40			
10/ 5/37	6.84	31			12.7	4.510	42
10/ 6/37					13.0	4.510	42
10/ 9/37	6.74	20	12.1	73 ¹			32(?)
10/11/37					13.0	4.600	
10/13/37	7.18	22	9.9	25	13.4	4.740	44
10/15/37	6.89	17	8.5	41			
10/16/37					13.4	4.600	44
10/19/37							42
10/21/37	5.88	20	11.4	40	13.4	4.630	42

¹ from formula $\frac{UV}{B}$. Other clearance values from $\frac{U}{B}\sqrt{V}$

Testosterone propionate given from 10/6/37 to and including 10/14/37. Although the testosterone propionate was given last on October 14th, the effect was still present on the 16th. Hemoglobin was determined by Newcomer, cell volume by hematocrit.

dose of 25 mg. of testosterone propionate² on 4 eunuchoids kept in the hospital on constant diets. Three of these men were alike in that the etiology of the condition was unknown, whereas the 4th had a suprasellar cyst, the hypogonadism, therefore, being of pituitary origin.

METHODS

The 4 patients were placed on weighed diets, constant in calories, carbohydrate, protein, fat, food water and minerals. The energy value was estimated so as to provide unchanging body weight, allowing for moderate, regulated activity during the day. This estimate was checked in patient *N. D.* by the measurements of insensible weight loss; in the others approximate checks were provided by the minimal variations in weight during the control periods. In 2, *F. R.* and *M. D.*, 3 alternating diets of similar composition, as judged by

² We are indebted to Drs. Erwin Schwenk, Gregory Stragnell, and Max Gilbert of the Schering Co. for generous supplies of testosterone propionate.

the standard food tables, were used; whereas in 2, *N. D.* and *H. S.*, the same food as nearly as possible was given each day. In *N. D.* the food was served hot and cold on alternate days, insensible weight loss being determined on the 'cold days.' Two gm. of added sodium chloride were given per day to each of the 4 patients. Two men (*F. R.* and *M. D.*) were available only in the

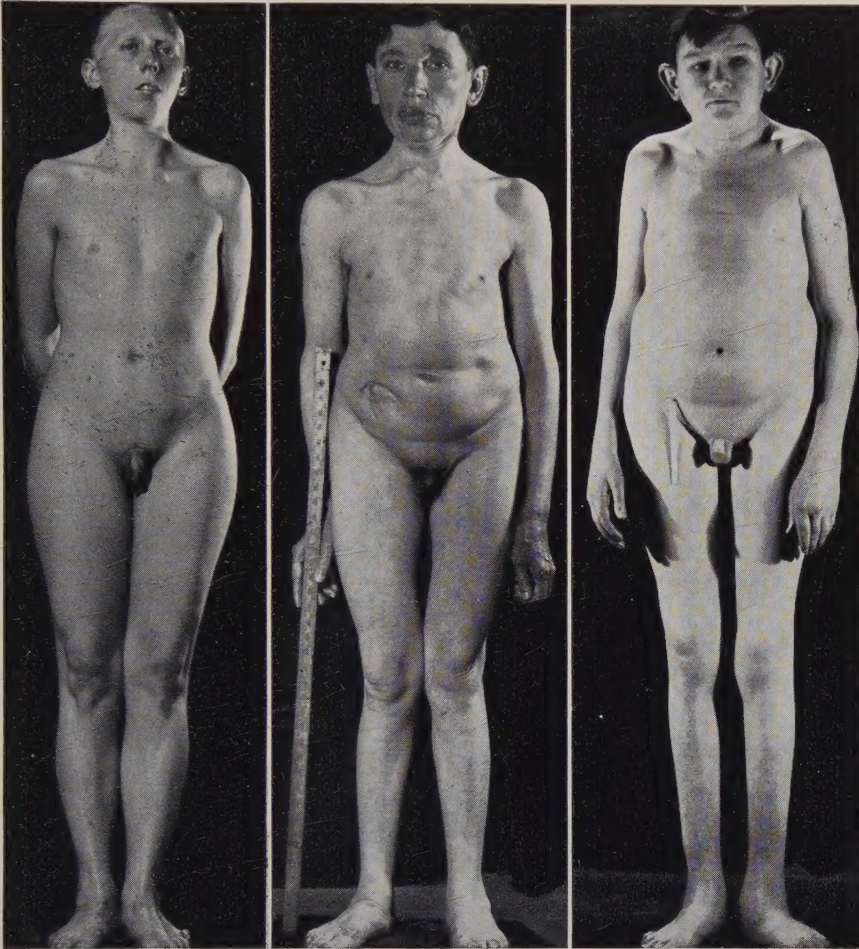


Fig. 1. Anterior views of *F. R.*, *M. D.* and *H. S.*, reading from left to right.
For *N. D.*, see preceding paper.

summer, and although sweating was minimized it must have occurred at times. The data on sodium and chloride are offered on these 2 only because they are in agreement with that in the others. *F. R.* and *M. D.* drank measured amounts of water as they chose, while *N. D.* and *H. S.* used fixed amounts.

The procedure for insensible weight loss was as follows. *N. D.* was weighed every morning on a chair balance which had a sensitivity of 5 gm. On alternate days the insensible loss of weight was determined in the usual way; all food, water, and excreta were weighed to the nearest gram. For each 24-hour period, the final body weight plus the weight of the excreta were

subtracted from the initial body weight plus the weight of the ingesta to obtain the insensible weight loss. Newburgh and his associates (17) have shown that the insensible loss of water, which accounts for about 90 per cent of the insensible loss of weight, is a function of the total metabolism. Thus, with care being taken to avoid visible perspiration, the measurement of the insensible weight loss serves indirectly as a gauge of the total activity of *N. D.* during the observation.

The urine for each day was kept in the ice-box at all times, and at the end of each 24 hours an aliquot was removed for chemical analysis and the remainder set aside for assay for sex hormones. In *F. R.* the urine was acid; in the first *M. D.* experiment it was neutral to alkaline, in the second *M. D.* experiment and in *N. D.* and *H. S.* acidity was insured by the addition of 10 cc. of 5 N sulphuric acid to the container. Except where otherwise noted, chemical determinations were made in the clinical chemistry laboratory of the Department of Medicine by procedures which Peters and Van Slyke (18) give in detail. Sodium in urine, Barber and Kolthoff; potassium in urine, Clausen, Kramer and Tisdall; chloride in urine, Volhard and Henry; creatine and creatinine in urine, Folin (creatinine by autoclave); urea N in blood and in urine by the gasometric urease method of Van Slyke; total N in blood and urine, macro Kjeldahl (19); plasma proteins, Campbell and Hanna (20).

Basal metabolic rate determinations in all were made by the Benedict-Roth and in *F. R.* and *M. D.* by the gasometer methods (21), and the Mayo Foundation standards (22) were used for comparison.

PROTOCOLS

F. R., aged 27 years (University of Chicago Clinics No. 113589) was referred by Doctors Carl Moore and C. B. Huggins. The eunuchoidism (fig. 1) was of uncertain origin, although a serious head injury and an infection or dermatitis involving the legs and scrotum in childhood presented possibilities of injury both to the pituitary body and to the testes. His standing height was 177.1 cm., sitting height 89.3 cm., sitting:standing ratio 50.4, greatest arm stretch 192 cm., ratio span:stature 108.4. His weight was 73.7 kg. and there was a considerable deposit of fat about the hips, giving a feminine contour to this region. Pubic, axillary and facial hair was scant, and his smooth face required no shaving. His voice was a rather high-pitched masculine one. The penis was from 5.3 to 6.4 cm. in length and 2.6 cm. wide at the corona. The testes measured 2.7 x 1.8 x 1.5 cm. Small prostatic lobes estimated as 4 mm. wide and 6 mm. long could be felt. Visual fields and x-rays of the sella turcica showed no abnormalities. X-rays on April 12, 1937 showed persistence of the line of epiphysial union at the head of the radius, from non-union to partial union of epiphyses with their corresponding metaphyses at the lower ends of the radius and ulna, bases of metacarpal I and proximal two rows of phalanges and heads of metacarpals II to V. Epiphyses about the knees were still incompletely fused. Those at the hip were fused. The delay may be estimated as about 6 years. A glucose tolerance after 72 gm. of glucose orally gave 65 mg./100 cc. fasting, 116 at $\frac{1}{2}$ hr., 33 at 2 hr. and 88 at 3 hr. He had a mild hypochromic anemia with a r.b.c. of 4,100,000, Hb. of 80 per cent (oxygen capacity = 16.5 vol. per cent), hematocrit showing 33 per cent cells. His urine was normal.

On May 27, 1937, the patient entered the surgical service of Dr. H. P. Jenkins,

and an appendectomy was performed for acute appendicitis. His convalescence was satisfactory. On June 9th he was placed on the dietary regime of C 204-211, P 75-78, F 108-116, Calories 2106-2196, after 4 preliminary days, in which the carbohydrate

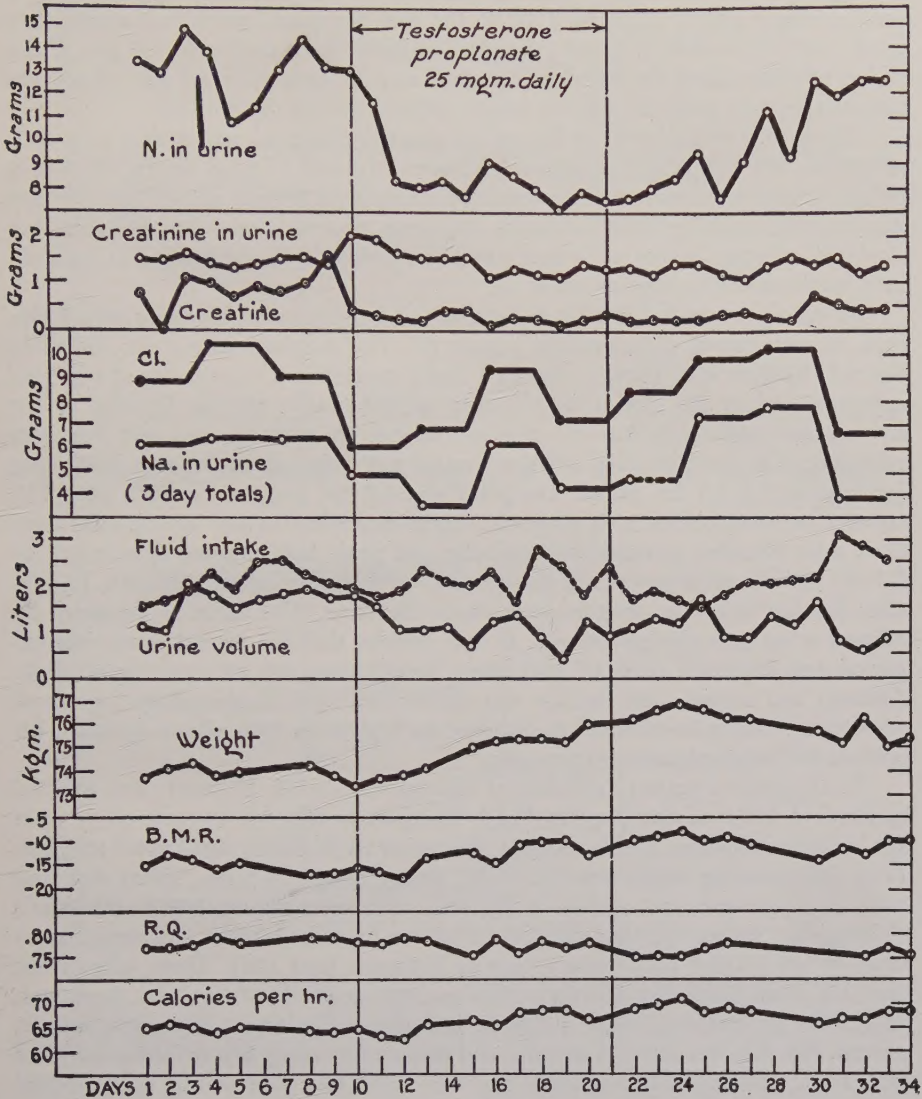


Fig. 2. The effect of testosterone propionate on F. R. This experiment extended from June 7th to and including July 10, 1937. Testosterone propionate was given from the 10th to and including the 21st day. The drop in urinary sodium and chloride and in urinary volume and increase in water intake, beginning on day 31, occurred during very hot weather. The dotted line for sodium at day 22 signifies that 2 analyses only were made for this 3-day period.

varied from 202 to 230 gm. per day, the other constituents remaining the same. Three diets of similar composition were alternated. As estimated from food tables he received daily from 2.4 to 2.9 gm. of chloride, from 1.3 to 1.9 gm. of sodium, from 3.0 to 4.1 gm. of potassium and from 1290 to 1390 gm. of water in his food. In addition, 2 gm. of sodium chloride were given as such. His water consumption

was unregulated. Certain changes to meet the taste of the patient and to provide him meat-less Fridays were necessary. On June 11th, he received 85.5 gm. of protein and 3.9 gm. of food chloride, on June 18th, 69.6 gm. of protein, on June 25th, 2.03 gm. of food sodium. On June 30th and July 3rd, the fat was increased to 130 gm., giving a caloric value of 2300 to the diet for those days. Two errors were made, for which allowances are possible. On June 9th he took only 1.4 gm. of the added salt instead of the standard 2.0 gm., and on June 16th 2.0 gm. of sodium bicarbonate were given by a house officer unfamiliar with the problem.

The patient was allowed to be up and about the hospital, and took a walk each afternoon. His weight was maintained between 73.4 and 74.3 kg. during the 9 days before testosterone, but he averaged a negative nitrogen balance of 0.59 gm. per day (estimated), omitting fecal nitrogen. Sweating was avoided when possible, but doubtless occurred. It was unfortunate that this patient had to be studied in the summer, but it was either then or not at all.

Between June 16th and 27th he received 12 intramuscular injections, of 25 mg. each, of testosterone propionate in sesame oil. The metabolic effects are shown in figure 2. By June 19th (day 13, fig. 2) a slight increase in the frequency of erections appeared, and by the 23rd (day 17) they became nearly constant. On day 18 the scrotum was sufficiently sore to cause discomfort on walking, and was markedly edematous. On the 26th (day 20) the prostate was definitely larger, each lobe being estimated at 1.0-1.5 cm. broad. The penis was 6.8 cm. long on the 28th (day 22) and 8.2 cm. long on the 30th (day 24). Eight days after the last injection the erections were recorded as subsiding gradually, the penis had returned to a length of 6.5 cm. and the prostate was still enlarged but questionably softer. July 10th, 12 days after the last injection, matters were much the same. The testes as measured by calipers at no time changed in size. It is of interest that this patient at no time admitted any improved sense of well-being, complaining not infrequently of slight dizziness and nausea, and that he was disconcerted and displeased by the sexual stimulation. When he returned to our care on September 29th after a vacation, his prostate had returned to the original size.

M. D., aged 51 years (University of Chicago Clinics No. 176188), was referred by Dr. S. J. Glass of Los Angeles, Calif. This patient (fig. 1) was seen many years ago by Falta in Vienna, and is shown at the age of 20 in Falta's well-known textbook (23). His standing height was 157.5 cm., sitting height 79.3 cm., sitting:standing ratio 50.3, greatest arm stretch 172.8 cm., ratio span:stature 109.7. Hrdlička's tables give a sitting:standing ratio of 52.76 for Jews in America and span:stature ratios of 103.3-103.8 for various groups of European Jews (24). These values illustrate the eunuchoid disproportion. This patient weighed 50 kg., but there was, despite the general emaciation, a definite pad of supra-pubic fat. Genu valgum was present. His skin was soft and atrophic and thrown into many fine wrinkles about the face. Pubic, axillary, limb and facial hair was scant. His voice was of fairly normal pitch but not deep, and was somewhat husky. The penis was 2.8 cm. long and 1.3 cm. wide at the corona. The testes were 2.1 x 1.3 x 1.2 cm. A small flat prostate could be felt. Visual fields were normal, but there was an enlargement of the blind spot in the left eye. The sella turcica was normal, and the epiphyses at the shoulder, wrist and hand, hip and knee were closed. There was considerable osteoporosis. Glucose tolerance was normal. The Wassermann and Kahn tests on the blood serum were negative, r.b.c. 4,000,000/cu. mm., Hb. 79 per cent (Sahli), w.b.c. 72,000, 53 per cent polymorphonuclears, 36 per cent large lymphocytes, 8 per cent small lymphocytes, 3 per cent mononuclears.

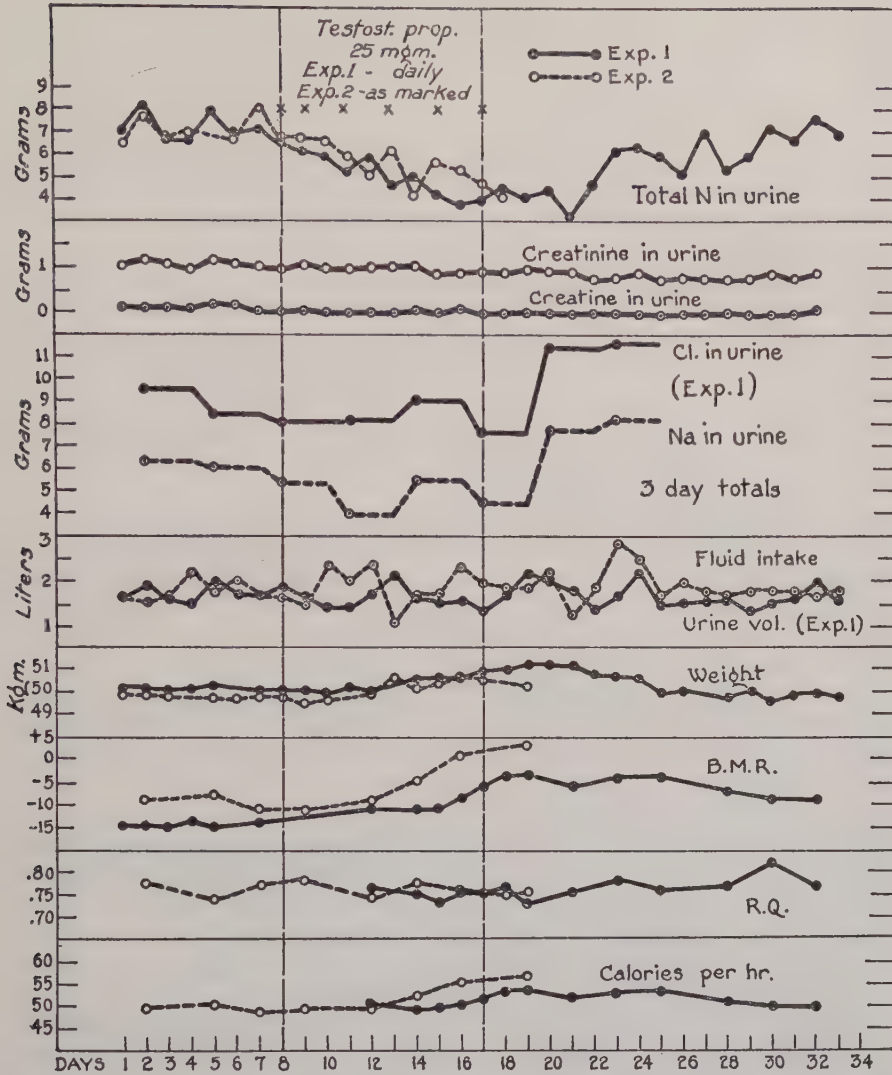


Fig. 3. The effect of testosterone propionate on *M. D.* The first experiment extended from July 13th to August 14, 1937, the second from August 12th to and including August 30th. The B.M.R. determinations were made by Benedict-Roth from day 1 to 7 of the first experiment, and thereafter, by the gasometer. Creatinine, creatine, chloride, sodium in the urine and fluid intake and urine volume are given for Exp. 1 only.

The patient was extremely nervous and anxious, and suffered greatly from a sense of inferiority. He had many visceral complaints, especially an epigastric burning and frequency and discomfort on urination. A bowel resection had been performed 9 years previously for tuberculosis of the colon. Dr. Glass had, in May 1936, demonstrated a diverticulum of the bladder by cystoscopic and urographic means. The mucosa of the bladder appeared normal except for inflammation at the posterior lip of the neck of the bladder. Urographic examination of the kidneys by skiodan intravenously showed no lesions. The examinations of Dr. Glass and our own repeated studies showed no abnormalities of the urinary sediment. X-ray studies of the

chest, gall-bladder, stomach, duodenum, and colon were normal at the University of Chicago, and many stool examinations were negative for blood. Nevertheless, the patient ran a slight fever (99° - 100°) much of the time, and this together with the sedimentation rate of 30 mm. in one hour suggested a low grade infection, the site of which was never ascertained.

The patient entered the hospital on June 20, 1937. On July 7, 1937, he was placed on the dietary regime of C 190-204, P 62-64, F 76-79, Calories 1718-1781. Three diets of similar composition were alternated. As estimated from food tables, he received daily from 1.93 to 2.13 gm. of chloride, from 1.81 to 1.93 gm. of sodium, from 3.32 to 3.53 gm. of potassium, in addition to an unknown but constant amount from the 30 gm. of 'Vitavose,' which he took daily. Food water varied from 1211 to 1267 gm. daily. Two gm. of sodium chloride were given daily as such. His water consumption was unregulated. One gm. of sodium bicarbonate was inadvertently given on July 18th and is allowed for in the excretion studies submitted. He received 30 minims of *Tr. Belladonna* daily, and between August 16th and 29th, 30 mg. of phenobarbital daily.

He was allowed to be up and about, and took a walk each afternoon. His weight was maintained between 50.0 and 50.3 kg. during the 7-day control period. Collections of urine were started on July 13th, and during the 7 control days he was estimated to be in positive nitrogen balance of 2.73 gm. per day, omitting fecal nitrogen. Sweating was avoided as much as possible. In this case, as in *F. R.*, the patient was available to us only in the summer.

Between July 20th and July 29th he received 10 daily injections of 25 mg. each of testosterone propionate in sesame oil. The metabolic effects are shown in figure 3. The evening of July 22nd (day 10, fig. 3) a great increase in the frequency of erections was noted. By day 16 the erections were constant, the priapism keeping the patient awake much of the night. It was not possible to measure the penis while it was at rest. By day 16 the prostate was not larger in area, but was much fuller than previously. This entire process excited the patient greatly, and the changes in B.M.R. recorded are perhaps questionable. Six days after stopping the testosterone propionate the erections began subsiding, and they had completely subsided by 8 days after. The psychic element here appeared large, and he was accordingly given 1 cc. of sesame oil every other day between August 10th and 18th, with the result that erections were again greatly stimulated.

On August 19th testosterone propionate, 25 mg. per dose, was given on alternate days to and including August 30th, 6 injections in all. On September 1st the observations were closed. The patient reported a great increase in erections during the last course of treatment, but no change in the prostate was observed.

N. D., eunuchoid, aged 31 years (University of Chicago Clinics No. 152001), was referred by Dr. M. Kolovros of Gary, Ind. (fig. 1). The record of the examination is given in the preceding paper. On September 19, 1937, he entered the hospital much the same as before his previous course of testosterone propionate. His penis was 7.2 cm. long and 2.8 cm. wide at the corona. The testes measured 2.5 x 1.6 x 1.4 cm. The prostate was very small and flat.

After 8 days on preliminary diets of approximately the same composition in carbohydrate, protein and fat, he was placed on a constant experimental diet of C 188, P 60, F 103, calories 1919, food water 1403. The same foodstuffs were used throughout, save that peas and asparagus were alternated, and mayonnaise substituted for part of the butter every other day. The vegetables and fruit were from the same case of canned goods, and 2 canned hams served as the meat, the second being

started on October 13th. The meals were prepared hot and cold on alternate days, the cold meals being used to permit accurate weighings for the determination of insensible weight loss, as described under the heading Methods. The butter was

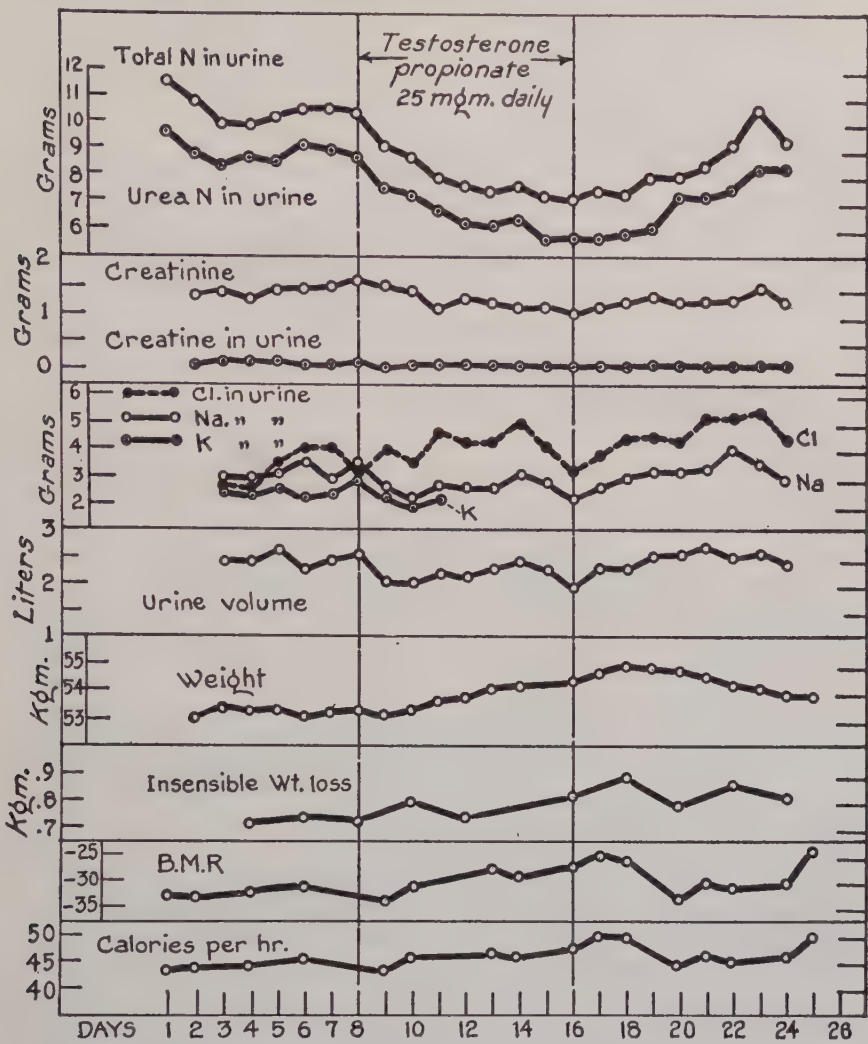


Fig. 4. The effect of testosterone propionate on N. D. extending from September 29th, to and including October 23, 1937. The fluid intake was constant, and included 1500 cc. of water daily.

salt-free, except on October 19th and 20th, when salted butter was served through an error. Analyses of the chloride content of the butter used permitted corrections in the mineral excretion on these days. The sodium content of the diet was 0.47 gm., chloride 0.89 gm., potassium 2.7 gm. in addition to that provided by the ham on which figures were not available. Two gm. of sodium chloride was provided daily as such. He drank 1500 cc. of distilled water daily, and the dishes used were washed in distilled water.

During the experiment the patient was allowed a fairly constant amount of ac-

tivity about the hospital and in daily walks outside. During the 7-day control period, from September 29th to and including October 5th, he maintained his weight between 53 and 53.3 kg., and was in an estimated negative nitrogen balance of 0.8 gm. daily, not counting fecal nitrogen. Between October 6th and 14th he received 9 daily intramuscular injections of 25 mg. each of testosterone propionate in sesame oil. On October 11th (day 13, fig. 4) erections were reported as increased, but the patient never emphasized this change and seemed reluctant to discuss the matter. The penis elongated slightly to 7.9 cm. on day 17, and resumed the original length of 7.1 cm., 6 days after stopping treatment. By day 14 (October 12th) the prostate had doubled in size, and 5 days after stopping the injections it had again reached the original size. The after-period lasted for 8 days until the morning of October 23rd. The metabolic effects are presented in figure 4 and in table 1.

H. S. eunuchoid, aged 24 (University of Chicago Clinics No. 186519) was referred by Dr. I. Becker of the University of Illinois (fig. 1). This patient was 171 cm. tall; his sitting height 77.1 cm., ratio of sitting: standing heights 45.1, the lowest in our series of 8 eunuchoids. Several skeletal deformities were present, including a severe pes planus of the left foot, limitation of extension of the forearms, due apparently to distorted growth of the olecranon, and limitation in abduction of the arms, not fully accounted for. The chest was narrow above, and flared toward the base. The epiphyses of the phalanges, metacarpals and distal ends of the radius and ulna were unfused, as was that of the head of the humerus, while that of the clavicle was closed. At the elbow the humeral epiphysis was closed, whereas those of the radius and ulna were still open peripherally. The pelvic epiphyses were not apparent except those of the Y-cartilages of the acetabulum which were not closed. The femoral neck was unusually long, the greater trochanter large, and the capital and trochanteric epiphyses were unfused. The patella was large and irregular, and the epiphyses about the knee were unfused except that of the tibial tubercle. The distal epiphyses of the tibia and fibula were unfused, the apophysis of the calcaneus had united to the calcaneus, the great toe was unusually long, the naviculars flattened and elongated, the epiphyses of the metacarpals were ununited. The delay in epiphyseal closure may here be estimated at fully 6 years.⁸ His weight was 64.3 kg., a suprapubic fat pad was present, and the abdomen was full and protuberant. There was no terminal pubic, axillary, limb or facial hair, the skin was smooth and boyish. His penis was 3.9 cm. long and 1.7 cm. wide at the corona. The scrotum was small and the testes 2.0 by 1.3 by 1.3 cm. The prostate could not be felt. Erections were rarely present, although the patient's story was variable in this regard. His voice was boyish, and the laryngeal prominence could not be felt.

X-ray of the skull showed an enormous dilatation of the sella turcica, with an antero-posterior diameter of 35 mm. and a depth of 15 mm. About 45 mm. above the sella were scattered patches of amorphous calcification, the area thus involved measuring 4 cm. in diameter. The optic discs were those of primary optic atrophy. There was no light perception in the left eye, and in the right eye vision was present only in the nasal field.

The Wassermann and Kahn tests on the serum were negative, r.b.c.: 4,470,000, Hb. 12.4 gm. (Newcomer), hematocrit 39 per cent, giving a mean corpuscular volume of 87.2 cu. micra and a mean corpuscular hemoglobin of 27.7, wbc. 7,500 with 61 per cent polymorphonuclears, 33 per cent lymphocytes, 4 per cent mononuclears and 2 per cent eosinophiles. A glucose tolerance test was normal.

⁸ We are indebted to Dr. C. H. Hatcher for his assistance in the analysis of the skeletal deformities.

On December 3, 1937, he was placed on a constant diet of C 197, P 64, F 100, Calories 1942, food water 1313. Vegetables and fruits from the same case were used and 3 sirloin butts, started respectively on December 3rd, December 19th and January 10th served as the meat. Salt-free butter was given throughout. As estimated from food tables, this diet contained 0.6 gm. of sodium, 0.9 gm. of chloride, and 2.7 gm. of potassium per day. Two gm. of sodium chloride was given daily as such. He

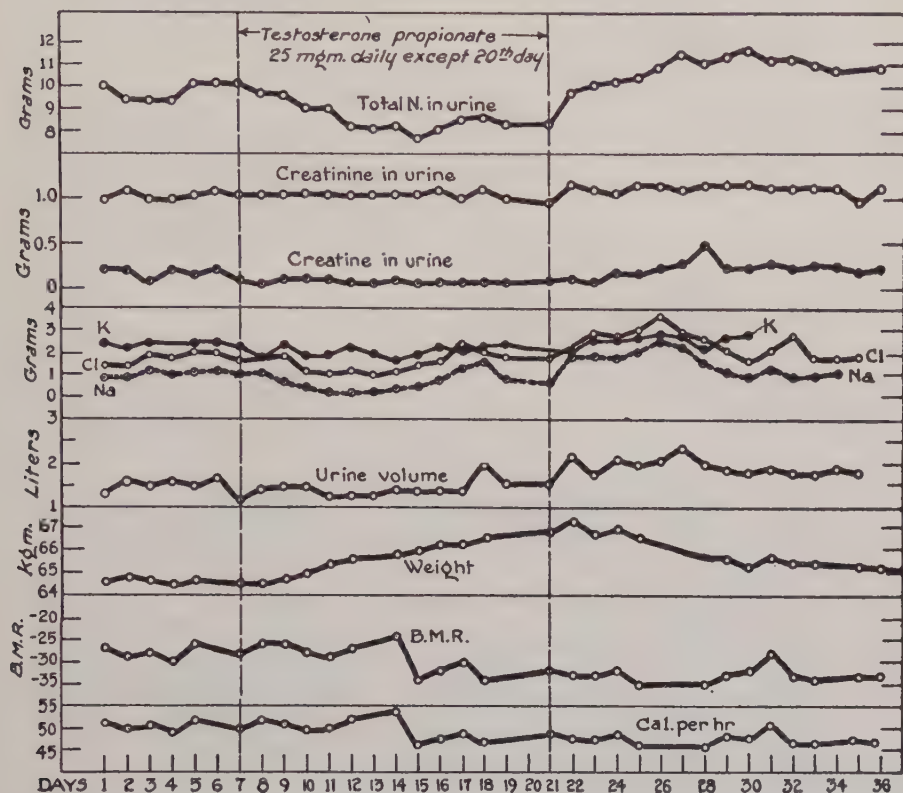


Fig. 5. The effect of testosterone propionate on H. S., who had a suprasellar cyst. The observations extend from December 7, 1937 to and including January 12, 1938. The fluid intake was constant, and included 1200 cc. of water daily.

drank 1000 cc. of distilled water on December 5th and 6th, and thereafter 1200 cc. daily. The dishes were washed in distilled water.

He was allowed to be up and about the hospital and, on occasion as weather permitted, walked outside. His weight was maintained between 64.4 and 64.8 kg. during the 8 control days, and he was in an estimated positive nitrogen balance of 0.4 gm. daily, not counting fecal nitrogen.

From December 13th to December 27th he received daily, 25 mg. of testosterone propionate intramuscularly, with the exception of December 26th, making 14 injections in all. Increased erections were noted by day 21 (fig. 5), but the patient volunteered nothing concerning these, and his testimony gave every evidence of uncertainty. The prostate by this time was, however, clearly definable as a thin sheet of tissue the area of a quarter. There was some edema of the scrotum, and the penis was 5.7 cm. long and 2.2 cm. wide, substantially larger than before. The testes were

TABLE 2. THE EFFECT OF TESTOSTERONE PROPIONATE ON THE URINARY EXCRETION OF NITROGEN AND ELECTROLYTES

	F. R.			M. D. (1)			M. D. (2)			N. D.			H. S.		
	Control	Exper.	Recov.	Control	Exper.	Recov.	Control	Exper.	Recov.	Control	Exper.	Recov.	Control	Exper.	Recov.
Average N Intake/day Average N in urine/day Estimated N balance/day ¹ Total N balance Total protein balance	9 da.	14 da.	7 da.	7 da.	11 da.	7 da.	7 da.	9 da.		5 da.	10 da.	7 da.	6 da.	15 da.	10 da.
	12.46	8.54	9.53	10.01	5.14	5.07	10.01	5.89		9.60	8.00	8.58	10.2	8.64	10.74
	13.05	+4.51	3.52	7.26	+2.12	+2.19	7.28	+1.39		10.39	+2.39	+1.81	9.8	+1.16	9.8
	-.59 ¹	+63.14	+94.6	+2.75 ¹	+23.3	+13.69	+2.73 ¹	+12.51		-.79 ¹	+149.4	+79.18	+4.1	+17.4	-9.40
Av. Na in urine gm./day Estimated Na balance gm./day Total Na balance (m. eq.)	2.09	1.54	2.51	2.02	1.64	2.51				3.04	2.69	3.29	1.06	.73	1.78
		+55	-.42		+.38	-.49					+.35	-.25		+.33	.72
		+7.70	-2.94		+4.18	-3.43					+3.50	-1.75		+4.95	-7.20
		+335	-128		+184	-149					+152	-76		+215	-313
Av. Cl in urine gm. per day Estimated Cl balance gm./day Total Cl balance gm. Total Cl balance (m. eq.)	3.15	2.48	3.40	3.16	2.90	3.64				3.60	4.01	4.76	1.78	1.58	2.66
		+87	-.26		+26	-.48					-.41	-1.16		+20	.88
		+0.38	-1.75		+2.86	-3.36					-4.10	-5.25		+3.00	-8.80
		+264	-50		+80	-95					-113	-148		+85	-248
Av. K in urine gm. per day Estimated K balance gm./day Total K balance gm. Total K balance (m. eq.)										2.60	2.25 (4 days)		2.36	2.09	2.55
											+.35				(7 days)
														+97	-.19
														+4.05	-1.90
Change in body weight in grams Change in body wt. from protein×4														+104	-49
														+2800	-1800
														+435	-235

¹ The estimated N balance during the control period is derived from the N in the urine and from the protein content of foods given in standard tables. Otherwise the estimated balances are obtained by comparison with the average excretion during the control periods. They are estimations only, and are not to be treated as exact values. The estimated columns cover the period from the beginning of testosterone propionate to the peak of weight gain. The 'recovery' columns cover periods for which data for the several constituents of urine are complete, and not the entire series of observations in all cases. The weight gained from protein is calculated on the assumption that three grams of water are stored with each gram of protein.

unaltered. Enuresis appeared the night of December 26th, spoiling this specimen, and some loss of urine with defecation occurred on December 27th. He was thereafter awakened repeatedly during the night and further significant losses prevented. Areas of wetting, 5 cm. in diameter, were present on his pajamas on January 1st and 4th, but inspection of the figure given presents no evidence that these losses were anything more than they appeared to be.

Following the completion of our studies, this patient was returned to the University of Illinois Hospital. Dr. Eric Oldberg attempted to deal surgically with the enormous cyst, but could remove only a few cubic centimeters of fluid, and the patient succumbed the second day thereafter. At autopsy an "enormous tumor was found which had destroyed the pituitary body and had tremendously distorted the optic chiasm and the circle of Willis."

DISCUSSION

In the eunuchoid, testosterone propionate consistently produces a striking drop in the urinary total nitrogen (fig. 2, 3, 4, 5), which is reflected completely in the urinary urea (fig. 4), and which is unaccompanied by any regular change in the urinary creatinine or by increase in the urea, non-protein nitrogen, plasma proteins or hemoglobin of the blood (table 1). The urinary nitrogen began to decline from the 1st to the 3rd day after starting testosterone propionate, reached minimum values from the 6th to the 13th days, and began increasing from the 1st to the 6th days after the last injection, approximating or exceeding the baseline from the 1st to the 10th days after the last injection. This effect is in entire agreement with the results of Kochakian and Murlin (13, 14) with urinary androgens and synthetic androstene-dione, and of Kochakian (15) with testosterone and testosterone acetate in the castrate dog. Indeed, the estimated nitrogen stored in our experiments, 0.02 to 0.06 gm. per kilo per day (table 2), precisely corresponds to Kochakian's for testosterone acetate. During recovery, the heightened nitrogen excretion exceeded the baseline only in *H. S.* (fig. 5), who lost all of his previous gain. *M. D.* (fig. 3) was observed for 21 days after the last injection of testosterone propionate without any signs of loss of the stored nitrogen. The Rochester workers, usually, but not always, observed a hypernormal excretion during recovery, but noted that part of the nitrogen gained could be tenaciously held for long periods.

The repository of the nitrogen stored in the body under the influence of the androgens remains uncertain. From 108.8 (*H. S.*) to 394.6 gm. (*F. R.*) of protein may be estimated as retained. The normal young adult prostate weighs 20 gm., and although it was clear enough, from our examinations that the prostate increased in size, it was equally clear that it never attained normal dimensions, and the same may be assumed for the seminal vesicles, which follow the prostate in normal development. Only a small fraction, not more than 10 gm., of the protein could go to form new tissue in the accessories. Some 5 gm. of nitrogen may be retained in the augmented extracellular fluid of the body (*N. D.*). The fate of the remainder is speculative. Korenchevsky, Dennison and Brovsin (5) have reported that testosterone increases the size of the somewhat smaller than normal livers, kidneys and hearts of castrated

rats, so that some new protein may well go to these organs. But consideration of such facts as the extraordinary muscular development in certain instances of precocious puberty suggests that muscle must also be considered as a possible site of new tissue formation.⁴

Fat may substitute in the metabolic mixture for the protein spared, as concluded by Kochakian and Murlin for the dog. Calculations in *N. D.* (fig. 6) from the total calories expended, urinary nitrogen, and carbohydrate intake (Newburgh, et al [17]) show a slight increase in fat consumption as the urinary nitrogen declines, as is inevitable from the assumption made that carbohydrate utilization equals carbohydrate ingestion. The fasting *R. Q.*'s given for *F. R.* and *M. D.* show little change. From calculations of the non-protein respiration quotient (after Lusk [25]) of *F. R.*, there is a slight increase in fat consumption as the nitrogen excretion declines, but such data is fragmentary from the standpoint of the metabolic mixture of the entire day, and unsatisfactory because of the nature of the respiratory quotient as a resultant of numerous opposing processes. Studies of nitrogen excretion after castration are entirely too fragmentary to permit comparison with the nitrogen-sparing effect of testosterone propionate. Korenchevsky (26) reported reduction of urinary nitrogen in the dog after castration, but his investigations were made some time after the operation. Kochakian and Murlin (14) could not obtain the nitrogen-sparing action of androstene-dione in the normal dog, a finding which indicates that this reaction is a genuine replacement effect.

We have obtained some evidence that creatinuria, when considerable in the eunuchoid living on a mixed diet, may be diminished by testosterone propionate. Thus, *F. R.* (fig. 2) averaged 844 mg. per day before treatment, 248 mg. per day during treatment, and showed a rise to between 420 and 670 mg. during the last 4 days of the recovery period. In *M. D.* (fig. 3), and *N. D.* (fig. 4), the spontaneous creatinuria was so irregular and slight (20 to 160 mg. per day) that it is difficult to feel sure of any influence of the androgen. In *H. S.* (fig. 5), the creatinuria was constant, and the drop from an average daily excretion of 165 mg. to an average of 65 mg. with a rise during recovery to 208 mg. is significant. As all of our patients except *M. D.*, who ate no meat, received considerable creatine in their diet, we can provide no information concerning the relative behavior of exogenous and endogenous creatine. The considerable literature which has grown up about the influence of the gonads on creatine metabolism cannot be reviewed here. Koch (27) has discussed some of this material, and pointed out the need for further careful study by better methods. The studies reported here, however, support the views of Bühler (28), Schittenhelm and Bühler (29) and others, to the extent that the androgens do modify creatine metabolism, and it is therefore entirely reasonable that their physiological secretion plays some part, at least, in diminishing creatinuria with the advent of adolescence, and that their absence is responsible for the occasional striking creatinuria of the eunuchoid.

⁴ Since this paper was written an important communication by Papanicolaou and Falk has appeared (35) in which it was observed that the temporal muscles of the male guinea pig are larger than those of the female, that castration reduces their size and that testosterone propionate causes hypertrophy of these muscles in the castrated immature male and in the spayed and normal adult female. The general muscle mass is also similarly affected. It is thus highly probable that some of the nitrogen stored in our eunuchoids went to the muscles.

The estimated retention of sodium (0.33 to 0.55 gm. per day), and of chloride (0.20 to 0.67 gm. per day) in all but *N. D.* (table 2, fig. 2, 3, 4, 5) produced by testosterone propionate is in agreement with the findings of Thorn and Harrop (6) that this androgen possesses a 'sodium-retaining' effect in the normal dog in common with its relatives, estrone, estradiol, pregnanediol, progesterone and the adrenal cortical hormone. The decline in urinary sodium was fairly well marked by the 3rd day, reached values of as low as a third of normal (*H. S.*) within the first 6 days, and was fairly well

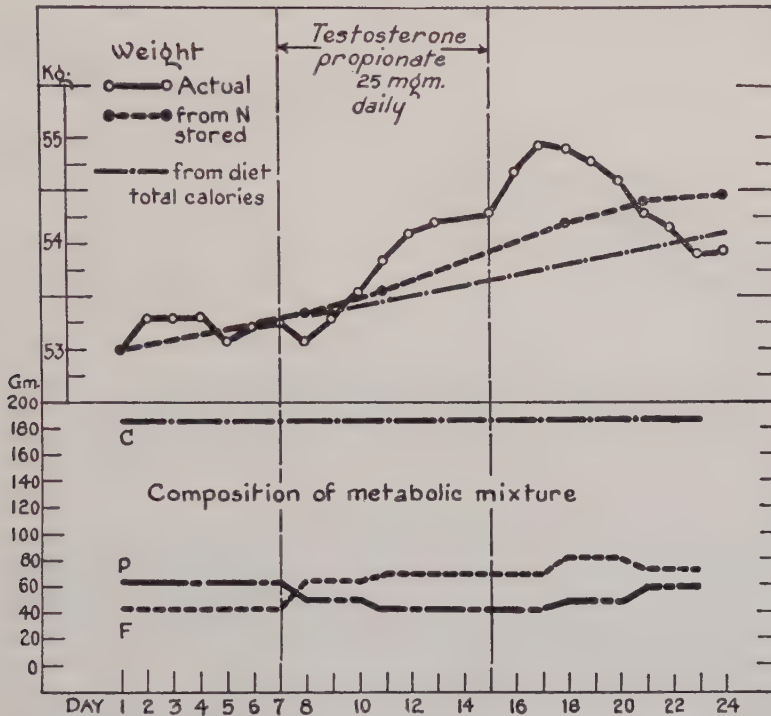


Fig. 6. Comparison of the actual weight gain in *N. D.*, with that expected from the nitrogen stored and with that predicted from the excess of diet calories over total calories expended. The curve of weight gain from nitrogen stored is calculated with the assumption that 5 gm. of water is deposited with each gram of protein stored. The total calories expended were derived from the insensible loss of water [calculated from the insensible weight loss, urinary N and carbohydrate of the diet after Newburgh et al (17)], and the third curve represents the gain in body weight which might be expected from the slight disproportion between diet calories and total calories, had not testosterone been given, and had the conditions of the control period been maintained throughout the observation.

The lower half of the figure illustrates the shift in metabolic mixture resulting from the administration of testosterone. The assumption is made that carbohydrate oxidized equals carbohydrate ingested.

sustained, although certain interruptions occurred. During recovery, the excretion of both sodium and chloride was sharply increased, and a hypernormal phase ensued, appearing from 1 to 6 days after the last injection. The rebound, which was more fully studied in *H. S.*, was of 10 days' duration, and resulted in the loss of slightly more sodium and considerably more chloride from the body than had been retained during the testosterone effect; estimations of 7.17 gm. of sodium and 8.79 gm. of chloride lost, as compared to 4.95 and 3.00 gained, respectively. It should be noted that the summer-time experiments

on *F. R.* and *M. D.*, which are only corroborative, show the same pattern and approximately the same quantitative results during the testosterone effect as the more satisfactory observations on *N. D.* and *H. S.* (table 2). A reduction in urine volume occurred with treatment, and a diuresis during recovery. Following the conceptions of Loeb (30) and Harrop (31) and their co-workers, concerning the mechanism of action of the adrenal cortical substance on electrolytes, it is probable that testosterone propionate likewise acts upon the kidney, but we can bring forward no direct evidence on this point. The failure to find increased potassium excretion (*N. D.*, *H. S.*) is of interest, as this commonly accompanies the 'sodium-retaining' action of the adrenal cortical hormone (7). Should this prove consistent, it may mean retention of potassium with nitrogen in new tissue, or the failure of testosterone to possess adrenomimetic properties in this regard. The possible physiological significance of these findings is completely unexplored. Inasmuch as Thorn and his co-workers (10) have found that changes in weight and in sodium excretion during the normal menstrual cycle may well depend upon the fluctuating secretion of the estrogens and progesterone, it would be unwise as yet to dismiss our observations as of pharmacological interest only.

The slight elevation of the B.M.R. in all instances except *H. S.*, who was hypopituitary, paralleling as it does the other more distinct reactions to testosterone propionate, is too consistent to be disregarded, although it is quite possible that subjective excitement rather than an immediate calorogenic influence is at work. This psychological influence seems especially likely in *M. D.*, who was greatly aroused by the sexual stimulation, and slept poorly. Our series of 8 eunuchoids (not including *H. S.*) showed an average initial B.M.R. of -11 per cent (range 0 to -19) by the Mayo Foundation Standards (21), whereas repetition to secure baselines for experimental purposes gave in 7 of these an average of -21 (range -14 to -32). As our only standards are based upon initial satisfactory tests, we must conclude that the eunuchoid has on the average a fairly normal basal rate, with a tendency, however, toward low values on occasion. McCullagh and Renshaw (32) in their series of 9 eunuchs, similarly record a range of $+5$ to -21 with an average of -13 . The slight rise, therefore, of 6 to 14 points after testosterone propionate is about the most influence that one could expect the testis to have on basal oxygen consumption. The careful studies of Kochakian and Murlin (13, 14, 15) produced evidence of such an increase in basal metabolism in only one of several animals studied, a fat castrate dog treated with urinary androgens. Two of our 4 patients, including *N. T.* (1), reacting in this way were thin, and 2 were of normal weight. Whether the differences between the Rochester group and ourselves lie in the greater excitability of man, or elsewhere, must be decided after longer experience with the problem.

Testosterone propionate produces a uniform gain in body weight of from 1.0 to 2.6 kg. per 10-day period when given in 25 mg. doses daily, with a subsequent decline during recovery. The magnitude of this change, obtained in those on a constant hospital diet, conforms to that secured in the out-patient department (1), in which food and activity were unrestricted (*J. H.*, 1.5 kg.;

H. K., 1.5 kg.; *N. T.*, 1.5 kg.; *N. D.*, 2.0 kg. per 10-day period). It is probable from this out-patient experience that, had hospital treatment been protracted, plateaus in the curves of weight gain would have been reached between the 40th and 65th days of treatment. The meaning of this phenomenon has been clarified to some extent. However much a factor increased appetite (*J. H.*, *H. K.*) may be in long experiments (1) when the food intake is unlimited, it is not necessary for the weight gain, nor is depression of the basal oxygen consumption important. Observations on *N. D.*, by the insensible weight loss technic, provide no evidence of a decline in total caloric expenditure. The retention of 17.4 to 63.1 gm. of nitrogen, built into 108.8 to 394.6 gm. of protein, and assumed to store 3 gm. of water for each gram of protein,⁵ provides for a gain in body weight of 435 to 1579 gm. (table 2). This would account for 1/7 (*H. S.*) to 1/2 (*F. R.*) of the total. The relation between the actual gain and the theoretical gain from protein stored, throughout the experiment on *N. D.*, is shown in figure 6. The fullness of the face, and the occasional obvious edema in the long experiments speaks for retention of extracellular fluid, and the decline in the urinary sodium and chloride supports this. Inasmuch as our experiments were designed to illustrate the general course of events and not to present exact electrolyte and water balances, we will not pretend to relate the mineral and water gains in a strictly quantitative fashion. Assuming, however, a concentration of 150 millimols of sodium per liter of retained water (34) for purposes of discussion, the 3.5 to 7.7 gm. of sodium estimated to be retained in our experiments provides associated water of from 1.0 to 2.2 kg., enough or more than enough to make up the difference between actual gain of body weight and that assumed to be due to the deposit of protein, except in *H. S.*, where it falls considerably (1 kg.) short. The recovery phases of the experiments presented attest further to the importance of salt and water in the weight attained, as in each instance weight was lost together with sodium, chloride and water, although nitrogen retention was still going on (*F. R.*, *M. D.*, *N. D.*) or nitrogen losses (as in *H. S.*) were insufficient to account for the decline.

It is of interest to compare the observations in the 3, in which the hypogonadism was of unknown etiology, with those in *H. S.* in which the defect was of pituitary origin. In general the results were the same, with the exceptions that the nitrogen stored was less in *H. S.* and the recovery excretion of nitrogen appeared more quickly and exceeded the baseline much more decisively, and that a slight decline in B.M.R. occurred rather than the slight increase. Whether these peculiarities of *H. S.* depend upon his hypopituitary state, or are variants of the normal influence of testosterone, remains to be determined by examination of a more extended series of patients.

In agreement with the results of the previous study, testosterone propionate produced increased frequency of erections and enlargement of the penis and of the prostate, with a return toward normal during recovery. It only remains to call attention to the fact that in the highly sensitive *M. D.*, plain sesame oil

⁵ The assignment of this particular value is made with full appreciation of its highly approximate character. Peters and Laviates (33) have thoroughly discussed these uncertainties. In any case, we lack information concerning the relative protein and water content of the tissues of the castrate.

alone increased the frequency of erections somewhat, at least as reported by the patient, thus emphasizing further the caution necessary in interpreting this reaction. Studies of the excretion of sex hormones during these experiments will be published later.

SUMMARY

Testosterone propionate, intramuscularly, in 4 eunuchoids, one of which was known to have pituitary disease, produced increased frequency of erections, enlargement of the penis and of the prostate; a consistent decline in urinary nitrogen, reflected in the urea fraction, without evidence of change in the nitrogenous constituents of the blood and amounting to a retention of from 1.16 to 4.51 gm. of nitrogen per day; a decline in urinary sodium, resulting in the retention of from 0.33 to 0.55 gm. per day, usually a retention of chloride; and a gain in weight due largely to the water held in association with the sodium and nitrogen. Creatinuria was diminished in 2 subjects, but the creatinine excretion was not consistently affected. The excretion of potassium declined somewhat in the 2 studied. A slight rise of 6 to 14 points in the B.M.R. occurred in 3 of 4 patients, and the respiratory quotient was unaltered in the 2 studied. Restoration toward normal occurred in all of the affected processes during recovery. Attention is called to the resemblances between the action of testosterone propionate on electrolyte excretion and that of certain of its chemical relatives among sex and adrenal hormones.

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A COMPARATIVE STUDY OF THE METABOLIC EFFECTS OF TESTOSTERONE PROPIONATE IN NORMAL MEN AND WOMEN AND IN EUNUCHOIDISM^{1,2}

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LAST YEAR, in part first of this thesis, we described our experiences with the metabolic effects of daily injections of testosterone propionate in 4 eunuchoids, one of whom had pituitary damage from a suprasellar cyst (1, 2). There appeared a uniform decline in urinary nitrogen reflected in the urea fraction and unaccompanied by increase in the plasma protein, non-protein nitrogen, urea or hemoglobin concentrations in the blood; a decline in urinary sodium associated, except in one instance, with a somewhat smaller decline in urinary chloride; a slight decline in urinary potassium in 2 experiments, of which one was fragmentary; a reduction in urine volume and a gain in weight due chiefly to water held with the retained electrolytes and nitrogen. Urinary creatinine remained unaffected while creatine declined in the 2 experiments in which spontaneous creatinuria was sufficiently intense and sustained to permit satisfactory study. The 3 patients without known pituitary disease, together with a fourth examined previously (3), showed slight rises in the basal metabolic rates. In conjunction with W. H. Hoskins and J. R. Coffman we showed that urinary androgens rose from subnormal levels to values usually within the upper reaches of the normal, and urinary estrogens, likewise increasing, attained the normal average (4). Within a few days after discontinuing testosterone propionate all affected processes moved well in the direction of the pretreatment baselines. It was pointed out that the amounts of protein estimated as stored, from 109 to 395 gm. in 10 to 15 days, were far in excess of those conceivably going to enlarging genital accessories, thus indicating deposit of new protein elsewhere in the body. The retention of sodium, chloride and associated water was held to be analogous to that produced by the chemically related adrenal cortical substances in men and dogs with intact adrenals (5-9), an analogy well supported by the demonstration of Thorn and Harrop (10), of the 'sodium retaining' properties of estrone, estradiol, progesterone, pregnandiol and testosterone and its propionate, all of the steroid family, in the normal dog. This analogy with the adrenal cortical substance failed, however, with respect to urinary potassium excretion since this usually increases after these steroids are administered to the normal organism rather than diminishing as in our eunuchoids after testosterone propionate. The dosage used in our work, 25 mg. of testosterone propionate daily, was quite sufficient in protracted

¹ The substance of this paper was read before the Association for the Study of Internal Secretions, May 12, 1939.

² This study was supported in part by grants from the Rockefeller Foundation.

experiments to cause enlargement of the penis and prostate, deepen the voice and increase the body hair of underdeveloped men (3).

It is now important to consider the response of the normal organism, already under the influence of its own androgens, to administered testosterone propionate. Kochakian and Murlin (11, 12) and Kochakian (13), who first described the nitrogen retaining properties of urinary androgens, androstene-dione and testosterone and its esters in the castrate dog, emphasized that there was a maximal effect in the neighborhood of 0.05 gm. of nitrogen stored per kg. per day which could not be exceeded by increasing or protracting the dosage. In one normal dog (12), they were unable to influence urinary nitrogen by 140 mg. of androstene-dione given in 3 portions in 4 days, the animal being followed for 11 days after the last injection. They considered, therefore, that the normal dog is at his maximum capacity for response by virtue of the influence of the hormones from his own testes. Thorn and Engel (14), however, have noted a decline in urinary nitrogen of as much as 4 gm. per day (0.4 gm. per kg. per day) after 25 mg. of testosterone propionate daily for 7 days in a normal dog. The discrepancies between these 2 experiments may lie in the androgen used, in dosage or in the state of gonadal function of the animals involved.

It now appears from the studies of Thorn and Engel (14) that the normal dog is quite insensitive to the 'sodium and chloride retaining' influence of testosterone propionate, daily injections of 25 mg. for 7 days producing no change whatever in the urinary excretion of these electrolytes or of water, although, as described above, substantial nitrogen retention did occur, accompanied by reduction in the urinary excretion of inorganic phosphorus and potassium. In an impotent man, otherwise normal (15), however, these workers (14) obtained a maximum reduction in urinary sodium excretion of about 30 m.eq. per day (ave. decline 19), a decline in urine volume and a gain in weight of 0.6 kg., together with a maximum decline in urinary potassium of about 7 m.eq. per day (ave. decline 5), a maximum decline in urinary inorganic phosphate of about 0.22 gm. per day (ave. decline 0.18) and a maximum decline in total urinary nitrogen of about 1.5 gm. per day (ave. decline 1.2), after giving 25 mg. of testosterone propionate intramuscularly for 6 days. The direction of these changes is in agreement with our experience in the eunuchoids, although the degree of change is in certain instances not as great, a point of some interest to be discussed at length later.

The present communication deals with an extension of our studies on the metabolic effects of testosterone propionate³ in eunuchoidism and a comparison of these effects with those obtained in 2 normal men and 2 normal women. One experiment on the effect of testosterone,⁴ by inunction, on a eunuchoid is included.

METHODS

The subjects were placed upon weighed diets containing the same food each day. The food was not analyzed, but reasonable constancy of intake was secured by (a) serving each individual slices from a single 20 lb. sirloin butt, which lasted about 25 days, supplemented by one or more such butts as necessary for the longer experiments; (b) serving the cooked foods and butter salt-free and adding measured amounts of salt at the table; (c) using canned fruits and vegetables largely, and these from the same case for each experiment, as far as possible; (d) by each patient eating all of his food each day. One to two weeks of the constant diet always preceded the beginning of collections. Caloric content was estimated to provide maintenance of weight with sufficient energy for a busy but regular day's work, as all of our normals continued their usual activities as students or as laboratory technicians, and our

^{3,4} The testosterone propionate and testosterone ointment were provided through the kindness of Drs. Gregory Stragnell, Erwin Schwenk and Max Gilbert of the Schering Corp., Bloomfield, N. J.

patient, J.X., occupied himself about the hospital and its environs. A. H. Bryan, in conjunction with ourselves, showed previously (2) that the characteristic effects of testosterone propionate are observed while the total caloric expenditure, as measured by the insensible loss of weight, remains essentially unchanged. The fluid intake was constant and sweating did not occur. In the second experiment on J.X. (fig. 1), 1.32 gm. of creatine hydrate (1 gm. of creatine) was given daily by mouth to insure sufficient creatinuria for study.

These conditions permit maintenance of daily urinary excretion of nitrogen with maximum deviations of 0.3 to 0.7 gm. from the mean, inorganic phosphorus with maximum deviations of 0.02 to 0.1 gm., and potassium usually with maximum deviations well under 10 m. eq.; but sodium and chloride may on occasional days deviate as much as 25 m. eq. from the average control value, without known errors in technic and without obvious explanation. Thorn and his coworkers (16) noted the same tendency for spontaneous and unexplained variations in sodium and chloride excretion in a normal untreated man.

For the most part, all of the urine passed was collected in containers to which just enough 5 N sulfuric acid had been added to insure acidity. In order to permit determinations of pH, etc., in the third experiment on the eunuchoid J.X., all urine reaching the bladder between 10:00 P.M. and 7:00 A.M. was voided at one time through a funnel, the stem of which dipped beneath oil in a clean cylinder. The remainder of the urine for these days was handled in the usual manner. During this experiment drinking water was given in specified amounts at specified times so as to minimize variations in urine volume during each period.

Analyses were made on the urine from each portion of the divided days of the third experiment on J.X., otherwise on 24-hour specimens, or as in the normal men, R.W. and J.L., on urine combined into 2-day lots. Chemical determinations were made in the clinical chemistry laboratory of the Department of Medicine by procedures given in Peters and Van Slyke (17), except where otherwise noted: sodium in urine and serum, Butler and Tuthill as modified by Eichelberger (18); chloride in urine and serum, Wilson and Ball; potassium in urine and serum, Shohl and Bennett as modified by Eichelberger (18);⁵ inorganic phosphorus in urine and serum, Fiske and Subbarow (19); urine pH by the glass electrode; total CO₂ in urine, Van Slyke manometric; titratable acidity of urine, Folin, to the endpoint of phenolphthalein; ammonia N in urine, Folin permuted with nesslerization; urea N in whole blood, gasometric urease method of Van Slyke; N.P.N. in whole blood and plasma, Koch and McMeekin as given by Koch (20); total N in urine and plasma, macrokjeldahl, distilling the ammonia formed into boric acid solution, according to the procedure described by Meeker and Wagner (21) and titrating to the brom cresol green endpoint; plasma proteins from (total N-N.P.N.) $\times 6.25$; creatine and creatinine in urine, Folin (creatinine by autoclave); cholesterol in whole blood, Lieberman-Burchard colorimetric determination in Bloor's alcohol-ether extract. Hemoglobin was determined by Newcomer and cell volume by hematocrit.

Basal metabolic rate determinations were made by the Benedict-Roth method on J.X. and E.S. and by the gasometer method (22) on P.J., R.W. and J.L. The Mayo Foundation standards (23) were used for comparison.

The androgen assays were performed in the Department of Biochemistry by W. H. Hoskins, J. R. Coffman and G. W. Beach and will be published in detail subsequently. Through the courtesy of these collaborators we are able to refer in our discussion to their results on the pooled urine during the control periods. The urine was kept in the refrigerator and extracted within the week following the termination of collections. After hydrolysis by boiling 15 minutes with hydrochloric acid, as described by Peterson, Gallagher and Koch (24), it was extracted with benzene in a continuous extractor, separated into androgenic and estrogenic fractions by partition between ether and 10% aqueous sodium hydroxide and the androgenic, ether-soluble fraction assayed on 7 capons by the method of Gallagher and Koch (25). The entire procedure is given in detail by Gallagher, et al. (26).

PROTOCOLS

J.X. (referred by Dr. Carl R. Moore), was a 27-year-old eunuchoid with bilaterally

⁵ This method was also used in our previous work instead of that of Clausen, Kramer and Tisdall as was erroneously stated in the paper (2).

TABLE 1. THE EFFECT OF TESTOSTERONE BY INUNCTION ON THE EXCRETION OF URINARY NITROGEN AND ON THE BODY WEIGHT OF THE EUNUCHOID, J.X. (FIRST EXPERIMENT)

Date	Procedure	Urinary N per day		Estimated N retention ¹		Change in body weight
		Average	Range	Per day	Total	
8/13 to 18	Control	gm. 7.63	gm. 7.04-7.94	gm. 0.59	gm. 5.31	kg. -0.3
8/19 to 27	Inunction (25 mg. testosterone daily)	7.04	6.60-7.38			-0.4
8/28 to 9/9	Inunction (50 mg. testosterone daily through 9/5)	6.33	5.36-6.94	1.30	15.60	+0.1
9/10 to 20	Recovery	6.75	5.61-7.26	0.88	9.68	-0.5
9/21 to 29	Control	7.42	6.79-7.84			-0.2

¹ The estimated retentions are based upon subtracting the average urinary nitrogen excretion for a given period from the average urinary excretion during the initial control period.

undescended testes who entered the Albert Merritt Billings Memorial Hospital August 2, 1938. Surgical treatment at the age of 9, and subsequent treatment with pregnancy urine extract had failed to effect descent of the testes. At the time of his admission he was 180 cm. tall and weighed 63 kg. His maximum weight had been 77 kg. 2 years ago. There was a supra-pubic fat pad and considerable deposit of fat about the hips giving them a feminine contour, but the breast areas had no unusual fat deposits. His face was smooth, boyish and devoid of terminal hair. Axillary hair was present but not abundant and pubic hair was scant. Limb and body hair was scant and fine. His voice was boyish and the laryngeal cartilage did not possess the characteristic masculine prominence. His testes could not be felt and the empty scrotum was small but well differentiated. The penis was 4.2 cm. long and 1.9 cm. wide at the corona. A very small amount of Y-shaped prostatic tissue was felt about the urethra. Erections occurred infrequently and sexual inclinations toward women were not recognized by the patient.

His initial B.M.R. was -28. X-rays showed a normal sella turcica. The epiphyses about the shoulder, elbow and hand were closed, those of the knee and distal ends of the radius and ulna were fusing. Visual fields were normal. The result of a glucose tolerance test was normal. Wassermann and Kahn test results were negative; Hb. was 13.2 gm. % (Newcomer);

TABLE 2. THE EFFECT OF TESTOSTERONE PROPIONATE, 25 MG. DAILY ON CERTAIN BLOOD CONSTITUENTS OF THE EUNUCHOID, J.X. (SECOND EXPERIMENT)

Date	Plasma proteins	N.P.N. of plasma	Urea N of whole blood	Hb.	R.B.C.	R.B.C.	Chol. of whole blood
	gm./100 cc.	mg./100 cc.	mg./100 cc.	gm./100 cc.	m./cu. m.m.	vol. %	mg./100 cc.
9/20/38	7.03	17	7.0	13.7	3.95	37	166
9/22	7.26	16	7.6	13.6	3.98	37	180
9/27	6.99	20	9.6	13.9	4.04	36	167
9/29	6.72	17	9.3	13.6	3.87	37	163
10/4	6.96	14	6.2	14.1	3.96	38	161
10/6	7.19	15	6.9	14.1	4.01	38	
10/11	6.74	15	6.2	14.4	4.10	38	155
10/13	6.16	11	4.2	14.1	4.01	38	
10/18	7.07	20	8.3	13.9	4.03	38	152
10/20	7.38	17	7.2	14.1	4.15	39	172
10/26	7.73	16	8.7	14.2	4.16	37	173
11/1	8.18	18	9.7				
11/3	7.26	18	8.8				178
11/8	6.72	19	9.8				161
11/11	6.94	20	11.0				157

Testosterone propionate was given intramuscularly from September 30 through October 13.

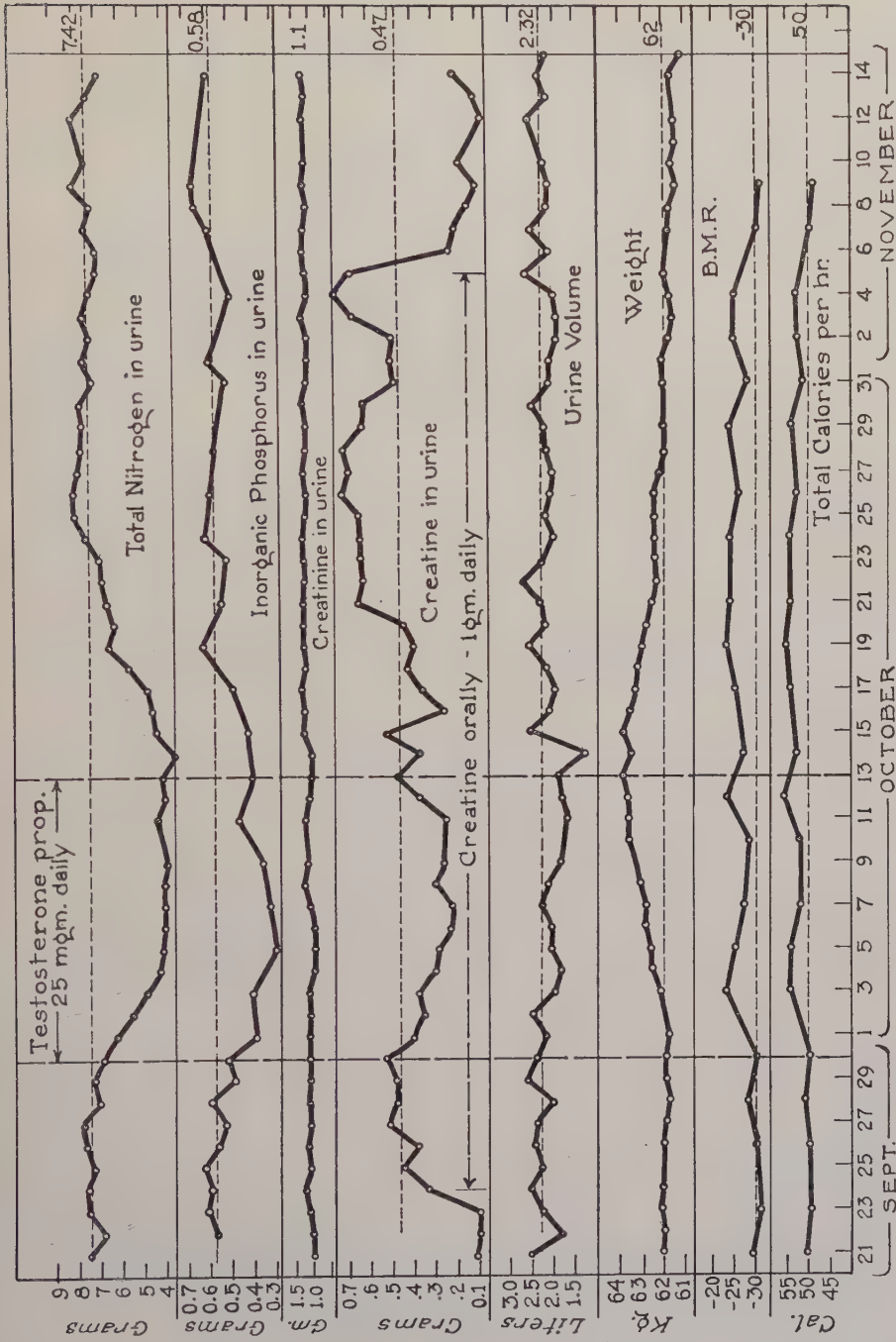


Fig. 1 THE EFFECT OF INTRAMUSCULAR INJECTIONS OF TESTOSTERONE PROPIONATE ON THE EXCRETION OF SEVERAL URINARY CONSTITUENTS, BODY WEIGHT AND B.M.R. OF THE BUNUCHOID J.X. (second experiment). Treatment was given from September 30 through October 13.

TABLE 3. THE EFFECTS OF TESTOSTERONE PROPIONATE (50 MG. DAILY), ON THE COMPOSITION OF THE NIGHT URINE OF THE EUNUCHOID, J.X. (3RD EXPERIMENT)

Date	Vol. cc.	pH	BHCO ₃		Titratable Acid		Inorganic Phosphorus		Ammono- nia N mM.	So- dium m.eq.	Chlo- ride m.eq.	Total N gm.
			mM.	mM.	m.eq.	m.eq.	mM.	mM.				
			period	liter	period	liter	period	liter	period	period	period	period
11/16	520	5.48	0.24	0.46	10.5	20.2	7.1	13.7	—	9.1	5.3	2.95
11/17	270	5.25	0.10	0.38	9.1	33.6	5.2	19.3	9	4.8	3.4	2.24
11/20	440	5.74	0.51	1.17	9.3	21.2	6.5	14.8	—	13.1	7.9	2.52
11/21	330	5.07	0.04	0.11	10.2	31.0	6.1	18.5	11	3.9	3.4	2.36
11/22	410	5.31	0.09	0.22	9.6	23.3	6.1	14.9	8	7.0	4.5	2.50
11/24	350	5.35	0.10	0.30	9.1	25.9	5.8	16.6	—	4.8	4.5	2.07
11/25	370	5.39	0.10	0.27	9.1	24.6	6.1	16.5	—	4.8	4.2	1.87
11/27	240	5.57	0.10	0.43	6.6	27.4	3.9	16.3	8	1.7	2.8	1.34
11/28	240	5.65	0.14	0.57	5.7	23.9	3.2	13.3	8	0.9	2.8	1.33
11/29	260	5.56	—	—	6.0	23.0	3.2	12.3	6	1.3	2.3	1.26
12/ 2	450	6.23	1.30	2.89	7.1	15.7	6.1	13.6	7	16.1	8.7	1.66
12/ 4	450	5.94	0.74	1.65	8.8	19.6	6.5	14.4	6	16.1	9.0	1.86
12/ 6	440	5.05	0.06	0.13	11.1	25.2	7.7	17.5	7	10.4	6.5	2.24

Testosterone propionate was given daily in 2 equal intramuscular injections from 11/23 through 11/27. The effects lasted some 3 days after the last injection. Each night period given represents urine for 9 hours, voided at one time in the morning. The creatinine excretion for this period was constant throughout the experiment. Titratable acidity is given to the endpoint of phenolphthalein.

r.b.c. 3,930,000 per cu. mm.; cell volume 38%; w.b.c. 4,700 per cu. mm., with a normal differential count.

Mr. John Evans of the Department of Biochemistry found about 8 mouse units of gonadotropic material in the benzoic acid precipitate of a 24-hour urine specimen tested upon the immature mouse.

TABLE 4. THE EFFECT OF TESTOSTERONE PROPIONATE (25 MG. DAILY) ON CERTAIN BLOOD CONSTITUENTS OF THE NORMAL MEN, R.W. AND J.L.

Date	N.P.N. in whole blood	Urea N in whole blood	P (inorg.) in serum	Na in serum	Cl in serum	K in serum
	mg./100 cc.	mg./100 cc.	mg./100 cc.	m.eq./liter	m.eq./liter	m.eq./liter
2/28/39	26		4.1	142.5	102.6	4.21
3/ 2	26		4.4	142.1	97.2	4.65
3/ 7	24		4.2	140.6	100.2	4.55
3/ 9	23		4.0	140.1	102.8	4.16
3/14	28		3.9	139.1	104.2	4.55
3/16	21		3.5	140.6	100.8	4.16
3/21	30		5.0	138.0	101.2	5.00
3/23	30		4.2		99.4	4.50
3/23/39		11.7	J.L.			
3/25		13.5	4.2			
3/30		11.5	3.8			
4/ 3		11.1	4.0			
4/ 6		11.8	3.7			
4/10		8.3	3.5			
4/14		11.8	4.5			
4/17		10.0	4.0			

Testosterone propionate was given intramuscularly from March 6 through March 15 in R. W., and from April 2 through April 9 in J. L. The effects were manifest for some two days after the last injection in each subject.

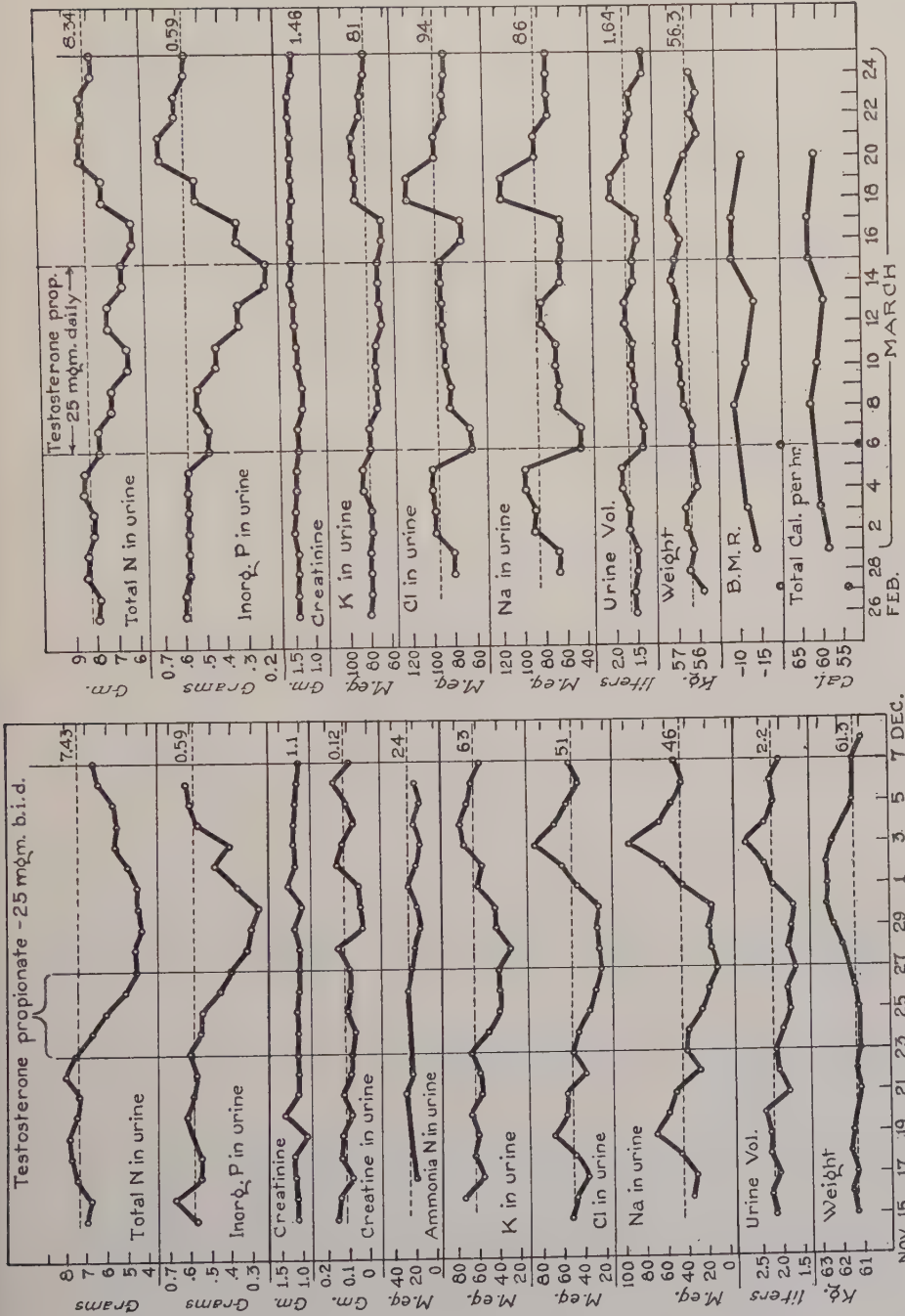


Fig. 2. (left). THE EFFECT OF INTRAMUSCULAR INJECTIONS OF TESTOSTERONE PROPIONATE ON THE EXCRETION OF SEVERAL URINARY CONSTITUENTS AND BODY WEIGHT OF THE EUNUCHOID J.X. (third experiment). Treatment was given from November 23 through November 27. Fig. 3 (right) THE EFFECT OF INTRAMUSCULAR INJECTIONS OF TESTOSTERONE PROPIONATE ON THE EXCRETION OF SEVERAL URINARY CONSTITUENTS, BODY WEIGHT AND B.M.R. OF THE NORMAL MAN R.W. Treatment was given from March 6 through March 15. The subject fell asleep during the determination of the B.M.R. on Feb. 27 and March 6, and the somewhat lower values for these days are hence recorded as isolated points.

On August 8 the patient was placed upon a constant diet of carbohydrate 267, protein 48, fat 100, Cal. 2,160, food water 1,580, drinking water 1,000, added salt 2 gm., giving an estimated content of N 7.7 grams, P 1.12 grams, Na 62 m. eq., K 83 m. eq., Cl 63 m. eq. as judged from standard food tables. He endured this diet with inexhaustible patience throughout the entire extent of his hospital stay. He was allowed to be up and about the hospital and campus each day.

Experimental observations were divided into 3 periods. From August 19 to and including September 5, testosterone in an ointment was rubbed into the skin of the trunk, chest and thighs, providing 25 mg. daily of testosterone from August 19 to 27, and 50 mg. daily from August 28 to September 5. In the 18 days of this experiment there was slight physical change.

TABLE 5. COMPARISON OF THE ESTIMATED REDUCTION IN EXCRETION OF SEVERAL URINARY CONSTITUENTS PRODUCED BY TESTOSTERONE PROPIONATE, GIVEN INTRAMUSCULARLY TO HYPOGONAD AND NORMAL MEN AND TO NORMAL WOMEN

Subject	Age	Condition	Urinary androgens before treatment	Amount of test. prop. given	Average reduction in excretion of urinary constituents						
					Total Nitrogen			Inorganic phosphorus	Sodium	Chloride	Potassium
					I	Maximal*					
						II	III				
			I.U. per day	mg. per day	gm./day	gm./day	gm./kg. per day	gm. per day	m.eq. per day	m.eq. per day	m.eq. per day
F.R.	27	Eunuchoid	13	25	4.51	5.14	.069		24	19	
M.D.	51	Eunuchoid	10	25	2.12	3.19	.064		17	7	
N.D.	31	Eunuchoid	3	25	2.39	3.03	.057		15	(-11)	9(4 dys only)
H.S.	24	Eunuchoid	2	25	1.16	1.59	.025		14	6	7
		Hypopituitary									
J.X.	27	Eunuchoid	29, 37	25	2.80	3.27	.053	.170			
	3	Eunuchoid		50	2.14	2.93	.048	.150	23	18	20
R.W.	21	Normal Man	83	25	1.37	1.66	.029	.190	21	15	9
J.L.	19	Normal Man	78	25	1.89	2.55	.030	.050	28	33	12
P.J.	24	Normal Woman	100	25	2.10	2.61	.043	.150	9	5	9
E.S.	31	Normal Woman	65	25	.56	.73	.016	.990	3	5	4

The values given for reduction in urinary excretion of various constituents are obtained by subtracting the average daily excretion during the 'experimental' period from the average pre-treatment level. The 'experimental' period thus used (excepting columns II and III under nitrogen) extends from the beginning of treatment to a point just before the onset of recovery, a point which occurs, as a rule, 2 or 3 days after the last injection of testosterone propionate.

* The 'experimental' periods covered in columns II and III under nitrogen extend from the time of approximately maximal effect of the androgen to just before the beginning of recovery. The data given for F. R., M. D., N. D., and H. S. are from previous publications (2, 4).

Toward the end he had several erections and the penis, then 5.0 cm. long, and the scrotum were slightly hyperemic. The prostate was unchanged. Data on nitrogen excretion and body weight are given in table 1.

One and thirty-two hundredths grams of creatine hydrate (1.00 gm. of creatine) were given orally in one dose daily from September 24 to and including November 5 to insure creatinuria. From September 30 to and including October 13 (14 days) he received 25 mg. daily of testosterone propionate intramuscularly in sesame oil. During the course of this treatment erections increased somewhat in frequency but were at no time as strong, frequent and troublesome as they may be, on occasion, with such medication. The hyperemia of the penis and scrotum returned and the penis increased in length from 4.2 cm. to 5.8 cm., and the corona in width from 2.1 to 2.5 cm.; the prostate seemed slightly larger to several observers. The metabolic data, accumulated during this study are given in figure 1 and table 6, and that on certain blood constituents in table 2.

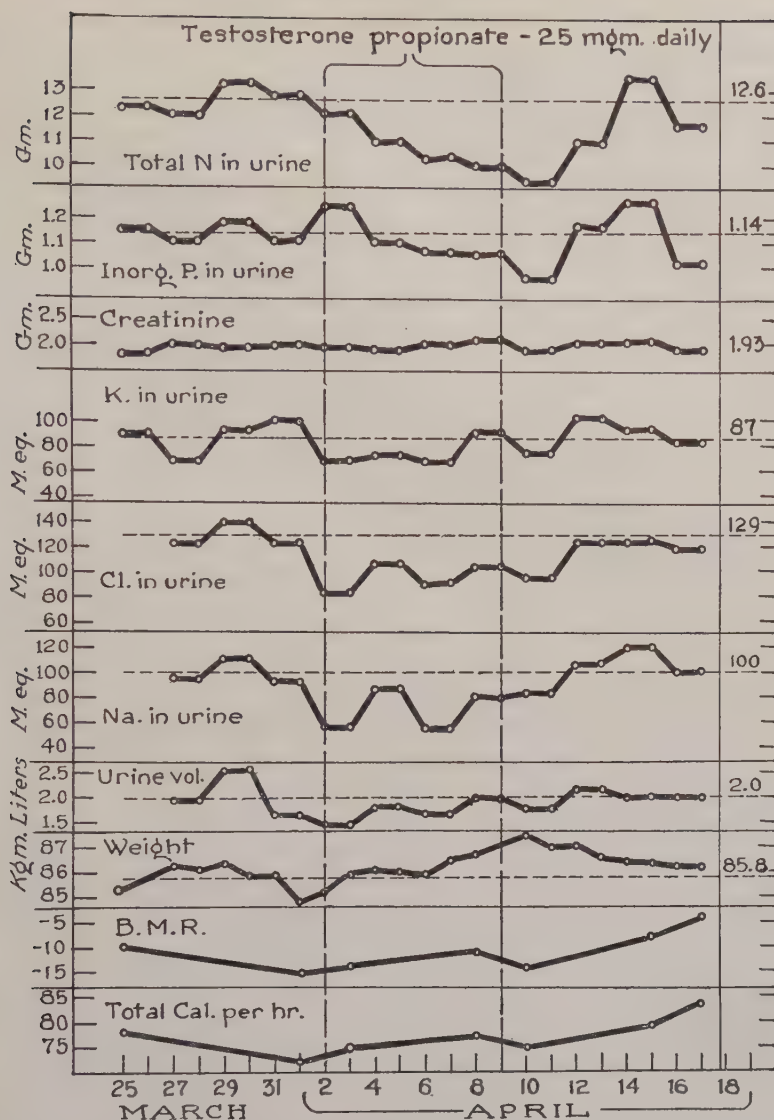


Fig. 4. THE EFFECT OF INTRAMUSCULAR INJECTIONS OF TESTOSTERONE PROPIONATE ON THE EXCRETION OF SEVERAL URINARY CONSTITUENTS, BODY WEIGHT AND B.M.R. OF THE NORMAL MAN J. L. Treatment was given from April 2 through April 9.

From November 23 to and including November 27 (5 days) he received 50 mg. of testosterone propionate, divided into 2 equal intramuscular injections in sesame oil. For several of the days of this experiment, each 24-hour period was divided into a night and day portion as described under methods. The data on the chemical studies of this urine are given in figure 2 and table 3.

R.W. and J.L. were normal young unmarried men, university students, age 20 and 19 respectively. They contrasted strongly in physical type. R.W. was of linear build and J.L. was heavy and broad. R.W. was blond and lightly bearded, requiring shaving only every other day, while J.L. was heavily bearded and had a deeper voice. The composition of the constant diets was for R.W.: carbohydrate 379, protein 60, fat 105, Cal. 2,705, food water

1,490, drinking water 1,000, added salt 4 gm.; with an estimated composition of N 9.6 gm., P 1.27 gm., Na 100 m. eq., Cl 100 m. eq., K 118 m. eq. For J.L. the diet was carbohydrate 443, protein 89, fat 143, Cal. 3,415, food water 2,070, drinking water 1,300, added salt 4 gm.; with an estimated composition of N 14.2 gm., P 1.76 gm., Na 112 m. eq., Cl 114 m. eq., K 121 m. eq. Both pursued their usual activities as students during the course of the experi-

TABLE 6. THE EFFECTS OF TESTOSTERONE PROPIONATE, GIVEN INTRAMUSCULARLY, ON THE B.M.R., FASTING RESPIRATORY QUOTIENT, PULSE RATE AND BLOOD PRESSURE OF NORMAL MEN AND WOMEN AND OF THE EUNUCHOID J.X.

Date	Weight kg.	B.P. syst./diast.	Pulse Rate	R.Q.	B.M.R.	Total Cal. per hr.	Date	Weight	B.P. syst./diast.	Pulse Rate	R.Q.	B.M.R.	Total Cal. per hr.		
R.W. Normal man, age 20, ht. 174 cm.							P.J. Normal woman, age 24, ht. 155 cm.								
2-20	55.0	104	68	66	0.75	-9	61.5	118	72	88	0.81	-1	56.5		
21	56.3	100	62	66	0.78	-8	62.8	113	61.6	114	72	86	0.74	-1	57.0
24	56.3	100	62	59	0.83	-16	57.3	14	62.0	114	68	82	0.78	-2	56.1
27	55.8	104	68	59	0.79	-20 ¹	54.4 ²	16	61.7	118	70	81	0.78	-5	54.2
3-1	56.3	104	68	65	0.83	-14	59.2	18	61.0	110	70	84	0.77	-2	56.0
3	56.6	106	58	68	0.86	-12	60.3	20	61.3	102	62	76	0.77	-9	52.0
							23	61.3	106	66	75	0.79	-6	53.7	
6	56.2	100	66	59	0.83	-22 ¹	54.0 ²	25	61.5	94	62	82	0.79	-6	53.6
8	56.6	102	60	70	0.81	-9	62.6	27	61.9	102	68	81	0.78	-5	54.2
10	56.8	102	66	67	0.82	-12	60.6								
13	56.9	92	50	65	0.82	-14	59.2	30	62.8	106	64	92	0.80	0	57.4
15	57.0	102	66	71	0.86	-9	62.5	2-1	62.9	102	60	84	0.83	-5	54.0
							3	62.8	108	66	87	0.80	-5	54.8	
17	57.2	104	64	69	0.83	-9	62.5	6	62.2	102	62	76	0.81	-3	55.7
20	56.4	102	60	66	0.84	-12	60.4	25 mg. testost. prop. daily Jan. 23-Jan. 29 inclusive. Menses 1-11 through 1-15, 2-2 through 2-5.							
25 mg. testost. prop. daily March 6-15 inclusive. ¹ Asleep?							E.S. Normal woman, age 31, ht. 165 cm.								
J.L. Normal man, age 19, ht. 181 cm.															
3-14	86.8	114	78	70	0.83	-3	83.8	1-13	45.9	94	60	58		-16	44.1
21	87.0	102	60	61	0.83	-12	76.3	14	45.4	92	54	55		-12	45.9
23	86.3	102	62	60	0.86	-16	72.7	16	45.3	88	50	54		-18	42.6
25	85.4	98	62	63	0.82	-9	78.5	17	45.3	94	58	55		-15	44.3
4-1	84.8	108	72	62	0.79	-15	72.6	18	45.1	90	52	55		-16	43.7
							20	45.4	84	48	57		-19	42.1	
3	85.9	102	68	60	0.83	-14	74.7	23	45.5	94	52	55		-17	43.4
8	86.7	102	66	67	0.85	-11	77.0	25	45.8	90	52	54		-18	42.8
							27	45.5	86	48	55		-14	45.2	
10	87.4	108	70	66	0.84	-14	75.2	30	45.8	94	60	54		-20	41.7
15	86.3	106	66	76	0.86	-8	79.5	2-1	46.2	96	56	58		-13	45.8
17	86.2	102	66	65	0.87	-4	83.3	3	46.8	90	56	59		-17	43.8
25 mg. testost. prop. daily March 2-April 9, inclusive.															
J.X. Eunuchoid, age 27, ht. 180 cm.															
9-21	62.1	102	62	44		-20	50.6	6	46.3	90	48	63		-15	44.8
23	62.1	102	62	45		-31	48.9	8	46.3	88	48	59		-14	45.2
26	62.0	102	72	43		-30	50.0	10	46.5	90	54	60		-21	41.7
28	61.8	102	72	45		-28	51.2	13	46.2	88	52	56		-22	40.6
30	61.9	102	62	45		-30	49.7	15	46.4	90	48	53		-21	42.0
							25 mg. testost. prop. daily Jan. 29-Feb. 4 inclusive. Menses 1-16 through 1-19 and 2-13 through 2-16.								
10-3	62.2	104	74	46		-23	54.6								
5	62.7	104	64	52		-25	53.9								
7	62.9	100	70	51		-27	52.3								
10	63.7	102	72	50		-28	51.9								
12	63.7	92	72	51		-23	55.6								
14	63.6	92	62	51		-27	52.5								
17	63.4	102	62	47		-25	53.0								
19	63.1	98	64	42		-23	54.8								
21	62.6	116	56	46		-24	54.0								
24	62.5	94	54	46		-24	54.0								
26	62.5	98	68	44		-26	52.7								
29	62.1	102	72	45		-24	53.9								
31	62.0	94	64	45		-28	51.6								
11-2	61.9	92	62	44		-25	51.6								
4	61.8	98	68	43		-25	53.3								
7	61.9	102	72	44		-30	50.0								
9	61.5	98	68	44		-31	48.8								

25 mg. testost. prop. daily Sept. 30-Oct. 13 inclusive.

ments. R.W. received 25 mg. of testosterone propionate daily in sesame oil intramuscularly, from March 6 to and including March 15, a total of 10 days. J.L. received 25 mg. of testosterone propionate similarly from April 2 to and including April 9, a total of 8 days. Neither was aware of any very distinct subjective response, although R.W. thought that erections might have occurred slightly more frequently and that a slight increased frequency of urination and tendency to contraction of the scrotal musculature were present during treatment.

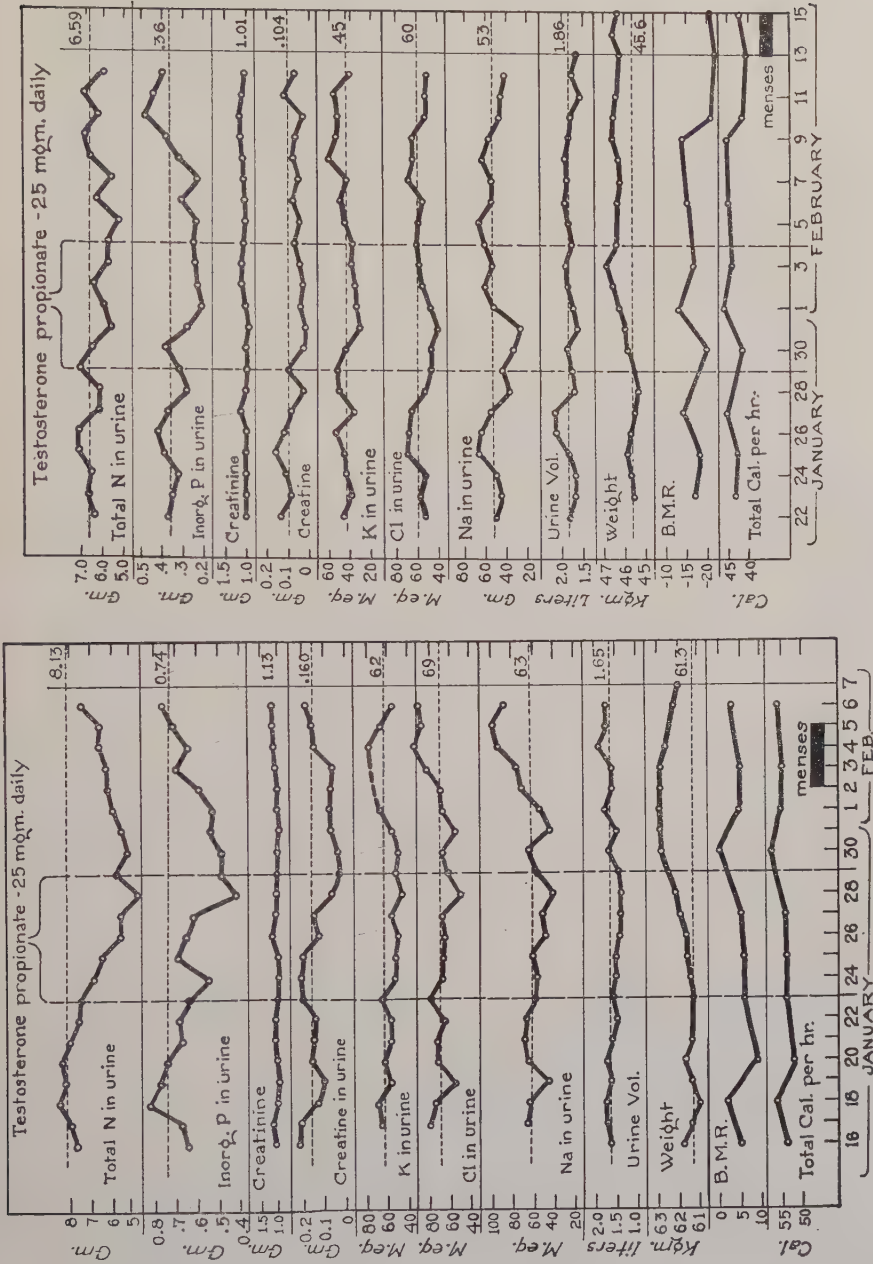


Fig. 5 (left). THE EFFECT OF INTRAMUSCULAR INJECTIONS OF TESTOSTERONE PROPIONATE ON THE EXCRETION OF SEVERAL URINARY CONSTITUENTS, BODY WEIGHT AND B.M.R. OF THE NORMAL WOMAN, P.J. Treatment was given from January 23 through January 29. The first day of the menstrual cycle studied was January 11. Fig. 6 (right). THE EFFECT OF INTRAMUSCULAR INJECTIONS OF TESTOSTERONE PROPIONATE ON THE EXCRETION OF SEVERAL URINARY CONSTITUENTS, BODY WEIGHT AND B.M.R. OF THE NORMAL WOMAN, E.S. Treatment was given from January 29 through February 4. The first day of the menstrual cycle studied was January 16.

Both men noted a slump in sense of well being about two days after stopping the injections, this being more conspicuous in *R.W.*, and more striking than any stimulation observed during treatment. The data on these subjects is presented in figures 3 and 4, and in tables 4 and 6.

P.J. and *E.S.* were normal young unmarried women, laboratory technicians, age 24 and 30 respectively. They contrasted physically, *P.J.* being short and plump while *E.S.* was taller and slender. The menstrual histories were as follows: *P.J.* began at 12, menses occurred every 28 to 30 days and lasted 4 to 5 days. *E.S.* began at 14, menses occurred every 32 to 35 days and lasted 3 to 5 days. Neither gave a history of a gain in weight before the onset of the menstrual flow and daily weighings before, during, and after the menses preceding collections showed no such gain in weight. The composition of the constant diets was for *P.J.*: carbohydrate 357, protein 54, fat 96, Cal. 2,508, food water 1,370, drinking water 1,000 cc., added salt 3 gm.; giving an estimated content of N 8.64 gm., P 1.11 gm., Na 79 m. eq., K 102 m. eq., Cl 78 m. eq. For *E.S.* the diet was carbohydrate 330, protein 44, fat 68, Cal. 2,108, food water 960, drinking water 1,000 cc., added salt 3 gm.; giving an estimated content of N 7.04 gm., P .62 gm., Na 65 m. eq., K 74 m. eq., Cl 60 m. eq. Both pursued their usual work in the hospital laboratories throughout the experiments.

The menstrual period of *P.J.* preceding the experiment began on January 11, 1939, and lasted through January 15. She received 25 mg. of testosterone propionate in 1 cc. of sesame oil intramuscularly on January 23, and thereafter daily through January 29, in all for 7 days. On the fourth day after the last injection a fully normal menstrual flow started and lasted for 4 days. This bleeding appeared, therefore, 22 days after the onset of the last preceding period or 6 to 8 days earlier than usual. The subject believed, however, that she probably had had such intermenstrual intervals on rare occasions in the past, and one such occurred in May, 1939.

Determination of the protein content of the urine collected during menstruation showed not more than one-tenth gram of protein nitrogen, so that the data on all constituents for these days is given without correction for contamination with menstrual blood. The subject had no subjective response of any kind to the treatment. The metabolic data is shown in figure 5 and table 6.

The menstrual period of *E.S.* preceding the experiment began on January 16, 1939 and lasted through January 19. She received 25 mg. of testosterone propionate in 1 cc. of sesame oil, intramuscularly, on January 29 and thereafter daily through February 4, for 7 days in all. The next menstruation started on February 13, or 28 days after the onset of the last preceding flow, and was entirely normal. This intermenstrual interval was only some 4 days shorter than usual for this subject. As in *P.J.* there were no subjective effects of the treatment. The metabolic data is shown in figure 6 and table 6.

DISCUSSION

The response of the eunuchoid *J.X.* to the injection of testosterone propionate is in agreement with our previous experience, summarized in table 5. The characteristic precipitate decline in urinary nitrogen excretion is well seen, proceeding at the same rate with the 25 mg. daily dose (fig. 1) as with the 50 mg. (fig. 2), and reaching a fairly stable minimum in about 5 days. Such cumulation of active androgen as may be provided by continuing the 25 mg. dosage for as long as 14 days is without effect in further depressing nitrogen excretion. Experiment has not as yet defined limits to the duration of this influence of testosterone propionate. The data on *J.X.*, however show that very considerable previous accumulations of nitrogen in no way interfere with a perfectly satisfactory demonstration of the nitrogen retaining effect. Thus, under the influence of testosterone injections (table 1), this patient retained an estimated 30 gm. in 33 days, added 55.4 gm. in 25 days as a result of the first course of testosterone propionate injections, lost only 3.5 gm. of this, and then gained 30.7 gm. further in 16 days during the second course with the larger dose of testosterone propionate by injection, making a net gain of 112.6 gm. of nitrogen, 703.8 gm. calcu-

lated as protein, in 4 months of discontinuous treatment and continuous observation. The inconspicuous losses of retained nitrogen during recovery are well illustrated by the data given for the after-period in figure 1 and the fore-period in figure 2, comprising in all 38 days following the last injection of 25 mg. of testosterone propionate. This, together with a similar 21-day period in the eunuchoid *M.D.* (2), attests to the tenacity with which the nitrogen compounds formed under the influence of the androgen may be held in the body. It should be noted that the amounts of nitrogen retained in *J.X.* during the periods of maximum effects of testosterone propionate are of approximately the same order as in the other eunuchoids, although the androgen excretion in the urine before treatment was substantially higher (table 5).

As in *N.D.* (2, table 1), the concentrations of plasma protein and non-protein nitrogen and blood urea nitrogen and hemoglobin are not increased during treatment. Indeed toward the later phase of the testosterone propionate effect reductions of 44 to 50% in the concentrations of the non-protein and urea nitrogen are observed on one isolated day. Similar reductions of 41 to 43% occurred in *N.D.* but were not stressed because they were unconfirmed at that time. Both constituents return towards normal during recovery in both experiments.

Moore, Lamar and Beck (27) observed that testosterone in ointment produced a powerful androgenic effect on the genital accessories of the rat when applied to the skin. In accordance with this, is the definite if slight, decline in urinary nitrogen produced in *J.X.* (table 1) with 25 mg. of testosterone applied to the skin daily and a more considerable decline with 50 mg. daily. There was no clear cut effect on body weight or on the prostate gland. Slight hyperemia and enlargement of the penis appeared toward the end of the experiment. In general the effects were much less than when the propionate was given intramuscularly, but as we have not tested the propionate by inunction direct comparisons are difficult.

The urinary excretion of inorganic phosphorus declines sharply after testosterone propionate (fig. 1, 2). The reduction of urine volume and of urinary sodium and chloride excretion and the gain in body weight in *J.X.* after 50 mg. of testosterone propionate (fig. 2), are of about the same magnitude as in previous experiments (2), (table 5) after 25 mg. daily, and the same sharp diuresis with loss of weight appears during recovery. Fortunately the reduction in urinary potassium, which was slight in previous experiments, is convincingly shown in figure 2, the estimated 'retention' reaching the considerable value of an average of 20 m. eq. per day. Although all of these various affected urinary constituents proceed more or less together during the development of the testosterone propionate effect, their behavior differs in an interesting manner during recovery. Thus sodium and chloride, deposited largely in extracellular spaces, are quickly lost shortly after treatment is stopped, while nitrogen, presumably stored as protein within cells, is still being retained. The excretion of inorganic phosphorus and potassium rises more rapidly to the baseline than does that of nitrogen, but, as would be expected from their intracellular deposition in the organism, these substances are not as rapidly or as completely discharged as are sodium and chloride.

The data on *R.W.* (table 4), a normal man, in the course of whose treatment much the same sequence of events took place as in the eunuchoids, show that the concentrations of sodium, chloride and potassium in the serum are unaffected during these times of changing electrolyte excretion. This is true, likewise, when adrenal cortical extracts are given to normal man (5). The concentration of inorganic phosphorus in the serum, however, in both *R.W.* and *J.L.* seems to decline somewhat towards the end of the testosterone propionate effect, to rise thereafter.

The fluid retained under the influence of testosterone propionate causes no blood

dilution, as evidenced by the constancy of plasma protein concentration, red blood cell count, and cell volume in *J.X.* (table 2), and in *N.D.* (2, table 1). Barring an as yet undemonstrated increase in circulating blood volume, this fluid, which makes up the greater part of the weight gain, is distributed between intracellular and extracellular extravascular compartments. More complete balance studies than we have at hand are required to assign the proper proportion to each repository.

In the normal dog Hartman, Lewis and Toby (8) have shown that ammonia excretion rises as sodium excretion declines after the administration of adrenal cortical extract, the pH of the urine remaining unchanged, and Stahl, Atchley and Loeb (28) reported falling ammonia excretion during sodium diuresis at the onset of suprarenal insufficiency in the dog. Such observations constitute the basis for the often quoted, but as yet inconclusively demonstrated theory of Jiménez-Díaz (29) that failure of ammonia formation by the kidney is an essential renal defect in suprarenal insufficiency and leads secondarily to sodium loss. It is of interest, therefore, that total ammonia excretion in *J.X.* (fig. 2, table 3) bore no reciprocal relationship to sodium excretion after testosterone propionate, remaining, indeed, pretty much unaffected throughout. With the declining urine volume, the constancy of total ammonia excretion per collection period makes for an increase in the concentration of ammonia during the androgen effect but this increase falls far short of compensating for the diminution in concentration of sodium. The pH of the night urine studied (table 3) remained unaltered during the androgen effect, just as in Hartman's dog, and the BHCO_3 content and concentration of titratable acid were unchanged. Thus the relative proportions of acid and basic phosphates may be considered constant, while the total phosphates excreted per collection period declined, largely accounting for the decline in total titratable acid. During recovery there was a brief excess of base loss with a transient rise in pH and BHCO_3 and a fall in the concentration of titratable acid.

In the previous experiments (2) we had some difficulty in demonstrating the effect of testosterone propionate on creatine excretion in the eunuchoid because of the frequently inconspicuous and irregular spontaneous creatinurias of these people. The constant feeding of 1 gm. of creatine daily to *J.X.* provoked an intense and fairly constant creatinuria (fig. 1), so that the effect of testosterone propionate in inhibiting creatine excretion is well shown. The excretion of creatinine is never changed in our experiments. A slight rise in basal metabolic rate appeared in *J.X.* after testosterone propionate (fig. 1), at approximately the same time as in several of our previous patients, but the decline during recovery was unusually delayed. Blood pressure and pulse rate were unaffected (table 6).

The two normal men, *R.W.* (fig. 3) and *J.L.* (fig. 4), show responses to testosterone propionate which are in most respects similar in kind to those of the eunuchoids. The actual amount of nitrogen retained per day during the time of maximal effect of the androgen is somewhat less in *J.L.* and distinctly less in *R.W.* than in any eunuchoid studied except *H.S.*, whose hypopituitarism presumably interfered with the deposit of protein in his tissues (table 5). The amount of nitrogen stored per kg. per day, during the maximal effect of the androgen, 0.029 gm. for *R.W.*, 0.030 gm. for *J.L.*, is about half that for the eunuchoids without gross pituitary damage. There is no greater tendency for the retained nitrogen to be discharged more quickly during recovery than in the eunuchoid.

While the inorganic phosphorus in the urine declines in both normals, the changes in *R.W.* are much the more decisive, the decline in this instance being as great as that in the eunuchoid *J.X.* with either the 25 or 50 mg. dose. Urinary sodium and chloride excretion drop sharply with the start of treatment and then rise to a variable

degree and for a varying length of time until the influence of testosterone propionate has waned, when the urinary excretion of both of these electrolytes rapidly increases. In *J.L.* this rising sodium and chloride excretion during recovery does not exceed the baseline in the decisive manner characteristic of this phase in all of our other experiments in which the androgen produced definite effects. Too much should not be made of the irregular character of the excretion of these substances under the influence of the androgen in contrast to the smoother, steadier decline in the eunuchoid *J.X.*, with the 50 mg. daily dose, because in previous studies in eunuchoids (2), the depression of sodium and chloride excretion was not infrequently discontinuous. The spontaneous daily variations in the urinary excretion of sodium and chloride which are manifest during control periods certainly contribute to these irregularities. Because of these same variations, estimates of the degree of reduction in excretion of these electrolytes are inexact but, as far as our data go, no differences are evident between our normals and our eunuchoids (table 5). Depression in urinary potassium excretion by an average of 9 m. eq. per day is convincingly shown in *R.W.*, with a subsequent resumption of baseline values after a brief supernormal phase during recovery. In *J.L.* irregularities of excretion blur the effect, but the same tendency is evident. The intensity of this influence on potassium excretion in these men is of about the same order as in the hypopituitary, *H.S.*, the eunuchoid *N.D.*, and the normal woman, *P.J.*, but considerably less than the 20 m.eq. reduction in excretion in the eunuchoid *J.X.*, with the 50 mg. daily dose of testosterone propionate. The gains in weight for these normal men after 8 days of treatment, *R.W.*, 0.8 kg., *J.L.*, 1.6 kg., fit well with those of the eunuchoids, which range from 1.0 to 2.6 kg. per 10-day period of treatment. No alteration in B.M.R., fasting respiratory quotient, blood pressure or pulse rate was produced by testosterone propionate in the amounts given (table 6).

As previously mentioned, the concentrations of sodium, potassium and chloride in the serum were unchanged during the course of the testosterone propionate effect, while that of inorganic phosphorus declined slightly to rise during recovery (table 4). Non-protein nitrogen of the whole blood in *R.W.*, however, and urea nitrogen of the whole blood in *J.L.* showed the same tendencies although with certain irregularities, toward lower values during the later phases of the testosterone propionate effect, with increases during recovery, that were apparent in the eunuchoids, *J.X.*, (table 2) and *N.D.* (2).

Before testosterone propionate injections, *R.W.* was excreting an average of 83 i.u. of androgenic activity per day for 8 days, and *J.L.* an average of 78 u per day for 6 days, both values being well up in the normal range (26, 30, 31). The natural androgens of these normal young men, however, are not exerting metabolic influences to the fullest extent to which the organism can respond. Indeed the effects of added testosterone do not differ in kind from those obtained in the eunuchoid, and distinctions in degree can thus far be made only with regard to the nitrogen retaining property. It is of interest that Thorn and Engel's impotent, but otherwise normal man (14) retained 0.022 gm. of nitrogen per kg. per day, not far from the 0.030 of our normals at a comparable time in the experiment. Our normal young men responded to testosterone propionate in a manner similar to Thorn and Engel's normal dog (14) and unlike the negative experience of Kochakian and Murlin (12) with androstenedione in their normal dog. Normal men of riper age, however may conceivably have a more intense secretion of testis hormone than our 20-year-olds and the effects of added androgens may be less accordingly. This remains to be determined. Age itself, between the years of 27 and 50, would appear to have little modifying influence on

these reactions, as the eunuchoid *M.D.*, who was 51, responded as well to the testosterone propionate as did younger eunuchoids.

The two normal women contrast strongly with one another in their response to testosterone propionate. The general course of the curve of nitrogen excretion in *P.J.* (fig. 5) was similar to those in the normal and hypogonad men. The average daily reduction in urinary nitrogen excretion during the time of maximal effect was 2.61 gm. (table 5), practically identical with that in the normal man *J.L.*, and only about 20% less than that of most of the eunuchoids. The 0.043 gm. of nitrogen estimated as retained per kg. per day fell between the 0.030 gm. of the normal men and the 0.060 gm. of the average eunuchoid without gross pituitary disease. *E.S.* (fig. 6), the second normal woman, on the other hand, showed a doubtful nitrogen retention, amounting to only 0.73 gm. per day during the time of maximal effect or 0.016 gm. per kg. per day, by far the lowest values in our series. The curve of inorganic phosphorus excretion in *P.J.* was much the same as in the normal man *R.W.*, or in the eunuchoid *J.X.*, and the degree of depression in urinary excretion was of the same order (table 5). This urinary constituent of all those studied in *E.S.* showed the most convincing evidences of a testosterone propionate effect. The average daily reduction in excretion of 0.090 gm. in *E.S.* was less than the corresponding 0.150 gm. in *P.J.* but this constituted a decline of 25% for *E.S.* as compared to one of 20% for *P.J.*

The decline in sodium and chloride excretion in *P.J.* under the influence of the androgen was slight and defined chiefly by the sharp diuresis of these constituents during recovery. No evidences of their retention were seen in *E.S.* Urinary potassium excretion declined somewhat in *P.J.*, and the constant pretreatment baseline, together with the restitution of these baseline values at the end of the experiment attests to the genuineness of this effect. The changes in *E.S.* are far too slight to have meaning. The gains in weight again show contrast between the 2 women. *P.J.* gained 1.6 kg. in characteristic pattern and was losing weight at the termination of the experiment, while the increase of 1.3 kg. in *E.S.* reached its peak by the 6th day of treatment, dropped off somewhat before the injections were stopped and remained, largely as a net gain of 1.00 kg., at the end of the experiment. Creatinuria seems to have been inhibited in *P.J.* by testosterone propionate, with a return towards the baseline during recovery. The changes in creatine excretion in *E.S.* are suggestive of the same influence in her, but the absence of the distinctive rise during recovery, together with the uncertainties surrounding the measurement of such small amounts of creatine as are involved, makes the experiment inconclusive. In neither patient were there significant alterations in oxygen consumption, fasting respiratory quotients, pulse rate, or blood pressure following testosterone propionate (table 6). Calculations of the non-protein respiratory quotient in *P.J.* showed a slight increase in carbohydrate and fat oxidized at the expense of protein, much as in the eunuchoid *F.R.* (2).

Thus, in the normal woman, the characteristic metabolic effects of testosterone propionate may appear in well defined form as in *P.J.*, or be slight, partial and often inconclusive as in *E.S.* The reason for the differences is not clear. The mature, menstruating woman is a difficult subject for metabolic experiments dealing with salt and water excretion. Spontaneous gains in weight, largely due to water retention are frequent just before menstruation or at the mid-interval and on rare occasions may result in gross edema (16, 32, 33). Thorn, Nelson and Thorn (16) observed declines in urinary sodium and chloride excretion and in urine volume to coexist with the weight gains in several subjects on constant food and fluid intakes, and attributed these phenomena to the salt retaining properties of the steroid ovarian hormones. Androgen excretion has certainly never been shown to vary in any periodic fashion

during the cycle (26, 35), and possible changes in the secretion of the adrenal cortical 'salt and water hormone' must remain a mystery for the present, so that the view of Thorn and his coworkers is entirely reasonable.

The effects of testosterone propionate in our subjects, therefore, may thus be superimposed upon those changes in sodium, chloride and water excretion that are likely to be proceeding in the body under the influence of its own hormones, and sharp distinctions between the influence of extrinsic and intrinsic hormones becomes difficult to draw. Neither of our 2 women, however, showed any spontaneous gain in weight immediately before the menstrual flow preceding our collection period, nor any loss of weight during or after the bleeding, so that a presumption exists against the weight gain in *P.J.* being a natural process. Furthermore by virtue of the unexpectedly early bleeding occurring in *P.J.* at 23 days, 4 days after stopping testosterone propionate, rather than at her usual 28 to 30 days, it is rather doubtful whether the premenstrual phase covered by the experiment was physiological or whether it represented merely a period of androgen influence preceding a spurious menses induced by testosterone withdrawal.

Nitrogen and phosphorus excretion show no periodic variations in the careful balance studies of Sherman, Gillett and Pope (32) on 3 cycles in 2 normal women, save for a drop in nitrogen excretion in the urine, the first day of menstruation, nor do Thorn and his coworkers (16) indicate any such variations in their subjects. Urinary potassium excretion appears from Thorn's work to be less strikingly involved in periodic electrolyte changes than do sodium and chloride excretion, although in one subject charted a slight increase appeared premenstrually. It seems legitimate, therefore, to conclude that the apparent effects of testosterone propionate on the urinary excretion of nitrogen, inorganic phosphorus and potassium, which were so conspicuous in *P.J.*, were real and not due to concurrent variations in the secretion of her own hormones. Similarly, the failure of *E.S.* to respond so clearly to testosterone propionate probably cannot be attributed to interferences with the experiment by her own endocrine secretions.

The slighter response of *E.S.* to the injected testosterone propionate cannot be explained by the theory that her organism was already under a greater load of natural androgens than was that of *P.J.* if we measure this load by the androgenic activity of the urine. Thus *P.J.*, who was excreting an average of 100 i.u. of androgenic activity per day for 8 days reacted well to injected testosterone propionate, while *E.S.*, who was excreting 64 u per day for 8 days reacted less decisively. More comprehensible correlations between natural androgen load and response to added androgens may become apparent, however, when our technics of urine assay provide for measurement of quantities of substances rather than total activities of mixtures of substances, and when the nature of the physiological androgens of the female and the course of their metabolic transformation into urinary representatives becomes better understood.

The nature of the changes in the tissues of the body induced by testosterone propionate can only be indicated in a rough and approximate way by such studies as ours, but enough has been learned to arouse our interest in them and our belief in their importance. It must be kept in mind that all of the effects discussed, with the exception of those on the male genitalia and their accessories may be indirect and involve the participation of other glands of the body to an as yet undisclosed degree. Testosterone propionate as far as it has now been studied in man and animals with intact adrenals, is adrenomimetic only in regard to diminishing urinary sodium, chloride and water excretion. Even in this function, the absence of a reciprocal rise in ammonia excretion suggests differences from the action of the adrenal cortical

hormone. Inorganic phosphorus and nitrogen excretion are not diminished by the adrenal hormones as they are by testosterone propionate, and potassium excretion is increased rather than reduced.

The amounts of nitrogen, calculated as protein, estimated as retained, from the beginning of treatment to the onset of the recovery period, came to from 102.7 gm. in 12 days for the normal man, R.W. to 252 gm. in 15 days for the eunuchoid J.X. (second experiment), all far in excess of any conceivable requirement of growing prostate and seminal vesicles, the changes in which even in the eunuchoid are barely detectable in such brief experiments. In the normal woman P.J. 117.9 gm. of protein were retained in 9 days. While Korenchevsky and Hall (36) have pointed out that protracted treatment of female rats with testosterone propionate will cause massive enlargement of the uterus and vagina, such was not the case in Zuckerman's experiments in the monkey (37), with doses much like our own; nor does it seem likely that any such 10-fold increase in the size of the uterus required to account for the protein deposit in P.J. could have occurred in the brief period of our experiment. We are thus left with the probability that non-genital tissues of affected normal women hold the important portion of retained nitrogen as do those of men.

We have already commented on the considerable reduction in the concentrations of blood urea nitrogen and N.P.N. that are observed toward the later phases of the testosterone propionate effect. These changes, which are demonstrably not due to blood dilution, are reminiscent of the depression in the concentration of these substances in the blood of the dog under the influence of the growth hormone from the anterior lobe of the pituitary body, as described by Teel and Watkins (38). During recovery the concentrations of these affected constituents again rise, the entire process being compatible with a retention of nitrogen in the tissues themselves during the androgen effect and release thereafter. Even if all of the 2 kg. weight gain induced by testosterone propionate in J.X. (second experiment) be assumed to be extracellular fluid, the nitrogen estimated as retained by virtue of expansion of this compartment amounts to not more than 5 gm. out of a total of 42 gm. for the period under discussion. Expansion of the volume of whole blood would, of course, make the amounts of circulating protein retained a more important fraction of the total, so that this point requires experimental study. Kochakian and Murlin's studies in the dog (11) indicate no unusual fecal nitrogen losses, accompanying the reduction in urinary nitrogen excretion.

The association of reduction in the urinary excretion of potassium and inorganic phosphorus with that of nitrogen is in accord with the conception of an augmentation of protoplasmic mass under the influence of testosterone propionate. However, we cannot quite yield to the temptation to speak of this as retention in the absence of studies of fecal excretion. The continued reduction in urinary potassium excretion of 7 to 20 m. eq. per day, in our experiments, nevertheless, cannot be accounted for by the increase in extracellular fluid or by a reasonable increase in volume of blood containing the usual 50 m. eq. of potassium per liter, so that, unless there is a compensatory fecal loss, potassium must be retained within cells. Similarly the continued reduction in urinary excretion of inorganic phosphorus of 90 to 190 mg. per day cannot be attributed to increase in extracellular fluid or to hypothetical expansion of the volume of blood, containing 390 mg. of total phosphorus per liter, so that here too cellular deposit must be invoked, unless a compensatory fecal loss can be demonstrated. When complete balance studies are available ratios of retained potassium to nitrogen and phosphorus to nitrogen will provide interesting information concerning the influence of androgens upon tissues. It is of especial importance in this connection that the recent careful balance studies of Sandberg, Perla and Holly (39) have dis-

closed reduction in the retention of nitrogen, sulfur, sodium, potassium and chloride after castration in the rapidly growing 7-week old rat. No such effect however appeared after castration of mature animals.

The evidences which we have adduced for a somatotrophic influence of the androgens in man, although incomplete in certain respects, provide a needed hypothesis for the interpretation of the striking if limited body growth and considerable muscular development that may accompany precocious puberty in man. Studies in animals which will be necessary for the more precise definition of these somatotrophic effects have just begun to appear. Thus Papanicolaou and Falk (40) have reported that castration reduces the size of the temporal muscles of the male guinea pig, and that testosterone propionate causes hypertrophy of these muscles in the castrated immature male and in the spayed and normal adult female. The general muscle mass also increased. Korenchevsky, Dennison and Brovsin (41) have reported that castration reduces the size of the heart, liver and kidneys of the male rat and that testosterone propionate restores the weights of these organs to normal. More extensive data of this kind is greatly needed. Thus far no really considerable growth of the body as a whole has been produced in animals by androgens.

SUMMARY

In confirmation of our previous studies, intramuscular injections of 25 mg. of testosterone propionate, daily or twice daily, in a eunuchoid, produced reduction in the urinary excretion of nitrogen, sodium, potassium and chloride, together with a gain in weight, due largely to water held in association with the salts and protein retained. A considerable reduction in the urinary excretion of inorganic phosphorus accompanied that of the other constituents. During recovery sodium, chloride and water were rapidly lost from the body, inorganic phosphorus and potassium less rapidly and less completely, while nitrogen stored was retained in great part for weeks.

Two normal young men responded like the eunuchoids, except that the 0.03 gm. of nitrogen retained per kg. per day, during the period of maximal effect, was half that of the eunuchoids.

One normal young woman responded essentially as the eunuchoids and the normal men. The second normal young woman showed a definite reduction in the urinary excretion of inorganic phosphorus but otherwise distinctly less evidence of a testosterone propionate effect. The reasons for the differences between these women are not clear.

The concentrations of urea and non-protein nitrogen of the blood were definitely reduced by such treatment, while those of hemoglobin, plasma proteins, and serum sodium, potassium and chloride were unaffected. Inorganic phosphorus concentration in the serum was doubtfully reduced.

The reductions in urinary electrolyte excretion, studied in the eunuchoid, were unaccompanied by important changes in urinary ammonia, pH, bicarbonate or concentration of titratable acid.

Creatine excretion, in the eunuchoid, intensified by ingestion of creatine, was substantially reduced by the androgen and increased during recovery.

Percutaneous administration of 25 and 50 mg. daily of testosterone itself in an ointment, in the eunuchoid, produced diminution in the urinary excretion of nitrogen.

The basal metabolic rate, fasting respiratory quotient, pulse rate and blood pressure of the normal subjects were not conspicuously affected by testosterone propionate. The basal metabolic rate of the eunuchoid was probably slightly increased; the pulse rate and blood pressure were unaffected.

The 102.7 to 252.2 gm. of protein estimated as retained by these subjects is not accounted for by increases in the bulk of genital tissues and represents deposit of new material elsewhere in the body. This together with the reduction in the urinary excretion of inorganic phosphorus, potassium and creatine points to a somatotrophic influence of androgens, and provides a clue to the explanation of unusual body growth accompanying precocious puberty.

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